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(54) Title: MELANIN CONCENTRATING HORMONE RECEPTOR CHIMERIC AND FUSION PROTEINS

(57) Abstract: The present invention features melanin concentrating hormone receptor (MCH-R) chimeric and fusion proteins. MCH-R chimeric proteins comprise an MCH-R polypeptide region made up of at least two or more polypeptide regions characteristic of MCH-R found in different species. MCH-R fusion proteins comprise an MCH-R polypeptide region and a fluorescent protein region.

TITLE OF THE INVENTION

MELANIN CONCENTRATING HORMONE RECEPTOR CHIMERIC AND FUSION PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority to provisional application U.S. Serial No. 60/189,698, filed March 15, 2000, hereby incorporated by reference herein.

BACKGROUND OF THE INVENTION

The references cited herein are not admitted to be prior art to the claimed invention.

Neuropeptides present in the hypothalamus play a major role in mediating the control of body weight. (Flier *et al.*, 1998. *Cell, 92*, 437-440.) Melanin-concentrating hormone (MCH) is a cyclic 19-amino acid neuropeptide synthesized as part of a larger pre-prohormone precursor in the hypothalamus which also encodes neuropeptides NEI and NGE. (Nahon *et al.*, 1990. *Mol. Endocrinol. 4*, 632-637.) MCH was first identified in salmon pituitary, and in fish MCH affects melanin aggregation thus affecting skin pigmentation. In trout and in eels MCH has also been shown to be involved in stress induced or CRF–stimulated ACTH release. (Kawauchi *et al.*, 1983. *Nature 305*, 321-323.)

In humans two genes encoding MCH have been identified that are expressed in the brain. (Breton *et al.*, 1993. *Mol. Brain Res. 18*, 297-310.) In mammals MCH has been localized primarily to neuronal cell bodies of the hypothalamus which are implicated in the control of food intake, including perikarya of the lateral hypothalamus and zona inertia. (Knigge *et al.*, 1996. *Peptides 17*, 1063-1073.)

Pharmacological and genetic evidence suggest that the primary mode of MCH action is to promote feeding (orexigenic). MCH mRNA is up regulated in fasted mice and rats and in the *ob/ob* mouse. (Qu *et al.*, 1996. *Nature 380*, 243-247.) Injection of MCH centrally (ICV) stimulates food intake and MCH antagonizes the hypophagic effects seen with α–melanocyte stimulating hormone (αMSH). (Qu *et al.*, 1996. *Nature 380*, 243-247.) MCH-deficient mice are lean, hypophagic, and have increased metabolic rate. (Shimada *et al.*, 1998. *Nature 396*, 670-673.)

MCH action is not limited to modulation of food intake as effects on the hypothalamic-pituitary-axis have been reported. (Nahon 1994. *Critical Rev. in*

Neurobiol. 8, 221-262.) MCH may be involved in the body response to stress as MCH can modulate the stress-induced release of CRF from the hypothalamus and ACTH from the pituitary. In addition, MCH neuronal systems may be involved in reproductive or maternal function.

Several references describe a receptor that is indicated to bind MCH. (Chambers *et al.*, 1999. *Nature 400*, 261-265; Saito *et al.*, 1999. *Nature 400*, 265-269; Bächner *et al.*, 1999. *FEBS Letters 457*:522-524; Shimomura *et al.*, 1999. *Biochemical and Biophysical Research Communications 261*, 622-626; and Lembo *et al.*, 1999. *Nat. Cell Biol. 1*, 267-271.)

SUMMARY OF THE INVENTION

The present invention features melanin concentrating hormone receptor (MCH-R) chimeric and fusion proteins. MCH-R chimeric proteins comprise an MCH-R polypeptide region made up of at least two or more polypeptide regions characteristic of MCH-R found in different species. MCH-R fusion proteins comprise an MCH-R polypeptide region and a fluorescent protein region.

An MCH-R polypeptide region provides a functional G-protein coupled receptor region able to bind MCH and transduce an intracellular signal. Examples of MCH-R polypeptide regions include naturally occurring MCH-R, chimeric MCH-R containing two or more regions from naturally occurring MCH-R, and functional derivatives thereof.

Reference to the terms "characteristic" and "derivatives thereof" describe a relationship to a reference sequence. In both cases, there is at least about 75% sequence similarity to the reference sequence.

Thus, a first aspect of the present invention describes a fusion protein comprising (a) an MCH-R polypeptide region and (b) a fluorescent polypeptide region. The fluorescent polypeptide region is joined directly, or though a polypeptide linker, to the carboxy side of the MCH-R polypeptide region.

Another aspect of the present invention describes an MCH-R chimeric protein. The protein comprises: (a) an MCH-R binding region characteristic of a human MCH-R, (b) a transmembrane domain characteristic of a non-human MCH-R, and (c) an intracellular domain characteristic of a non-human MCH-R.

Another aspect of the present invention describes a nucleic acid encoding for an MCH-R fusion protein or an MCH-R chimeric protein described herein. Such nucleic acid comprises either a contiguous nucleotide sequence that

codes for the protein or a sequence that is processed by a host cell to produce a contiguous nucleotide sequence encoding for the protein. Processing of a nucleic acid sequence to produce a contiguous nucleotide sequence encoding for a protein can occur by the splicing together of exons resulting in intron removal.

Another aspect of the present invention describes an expression vector comprising a nucleic acid encoding for an MCH-R fusion protein or an MCH-R chimeric protein described herein.

Another aspect of the present invention describes a recombinant cell comprising nucleic acid encoding for an MCH-R fusion protein or an MCH-R chimeric protein described herein. The nucleic acid may be part of the host genome or may exist independently of the host genome.

Another aspect of the present invention describes a non-human transgenic animal comprising nucleic acid encoding for an MCH-R fusion protein or an MCH-R chimeric protein described herein.

Another aspect of the present invention describes a method for assaying for MCH-R active compounds by measuring the effect of a test preparation on one or more MCH-R activities. The method is performed using either an MCH-R fusion protein or an MCH-R chimeric protein described herein.

Other features and advantages of the present invention are apparent from the additional descriptions provided herein including the different examples. The provided examples illustrate different components and methodology useful in practicing the present invention. The examples do not limit the claimed invention. Based on the present disclosure the skilled artisan can identify and employ other components and methodology useful for practicing the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates aequorin assay results comparing a mouse MCH-R fusion with a human wild type MCH-R and a CMV-EGFP control.

Figure 2 illustrates a cAMP flashplate assay of CHO cell clones stably expressing mMCH-1R-EGFP. Cells from individual clones were dissociated in enzyme free media and stimulated for 15 minutes at 37°C with human MCH at the indicated concentrations in the presence of 10 μM forskolin. Cells were then lysed and assayed for bound [^{125}I]cAMP. Mouse MCH-1R-EGFP clones exhibited EC50 values (0.1111, 0.1255, 0.1291, or 0.2304 nM) indistinguishable from that of a CHO cell clone expressing the wild-type human short isoform of MCH-1R (0.1282 nM).

Figure 3 illustrates a cAMP flashplate assay of CHO cell clones stably expressing human short/mouse species chimeric MCH-1R-EGFP. Cells from individual clones were dissociated in enzyme free media and stimulated for 15 minutes at 37°C with human MCH at the indicated concentrations in the presence of 10 μM forskolin. Cells were then lysed and assayed for bound [^{125}I]cAMP. Human short/mouse species chimeric MCH-1R-EGFP clones exhibited EC50 values (0.0366, 0.0462, 0.2117, or 0.2499 nM) indistinguishable from that of a CHO cell clone expressing the wild-type human short isoform of MCH-1R (0.1137 nM).

DETAILED DESCRIPTION OF THE INVENTION

The present invention features MCH-R chimeric and fusion proteins. Such proteins have a variety of different uses including being used as a research tool to study MCH-R function and dynamics, and being used to screen for MCH-R agonists and antagonists.

The MCH-R provides a target to achieve different beneficial effects in a patient. Preferably, MCH-R activity is modulated to achieve one or more of the following: weight loss, weight gain, treat cancer (*e.g.*, colon or breast), reduce pain, treat diabetes, reduce stress, or teat sexual dysfunction.

Modulation of MCH-R activity can be achieved by evoking a response at the MCH receptor or by altering a response evoked by an MCH receptor agonist or antagonist. Compounds modulating MCH-R receptor activity include agonists, antagonists, and allosteric modulators. Generally, MCH-R antagonists and allosteric modulators negatively affecting activity will be used to achieve weight loss, treat cancer (*e.g.*, colon or breast), reduce pain, reduce stress, or teat sexual dysfunction; and MCH-R agonists and allosteric modulators positively affecting activity will be used to produce a weight gain.

Preferably, MCH-R activity is modulated to achieve a weight loss or to treat diabetes in a patient. Diabetes mellitus can be treated by modulating MCH-R activity to achieve, for example, one or both of the following: enhancing glucose tolerance or decreasing insulin resistance.

Excessive body weight is a contributing factor to different diseases, including hypertension, diabetes, dyslipidemias, cardiovascular disease, gall stones, osteoarthritis, and certain forms of cancers. Bringing about a weight loss can be used, for example, to reduce the likelihood of such diseases and as part of a treatment for such diseases. Weight reduction can be achieved by modulating MCH-R activity to

obtain, for example, one or more of the following effects: reducing appetite, increasing metabolic rate, reducing fat intake, or reducing carbohydrate craving.

Increasing body weight is particularly useful for a patient having a disease or disorder, or under going a treatment, accompanied by weight loss. Examples of diseases or disorders accompanied by weight loss include anorexia, AIDS, wasting, cachexia, and frail elderly. Examples of treatments accompanied by weight loss include chemotherapy and radiation therapy.

MCH-R Chimeric Proteins

MCH-R chimeric proteins contain an MCH-R polypeptide region made up by at least two or more polypeptide regions characteristic of MCH-R found in different species. The different polypeptide regions that are present provide for an N-terminal extracellular domain; a transmembrane domain made up of transmembrane regions, extracellular loop regions, and intracellular loop regions; and an intracellular carboxy terminus domain. Examples of MCH-R amino acid sequences include the following: SEQ. ID. NO. 1 (human MCH1R long form), SEQ. ID. NO. 2 (human MCH1R short form), and SEQ. ID. NO. 3 (mouse MCH1R).

Preferably, the MCH-R chimeric protein comprises an MCH-R binding region characteristic of a human MCH-R along with transmembrane and intracellular domains characteristic of a non-human MCH-R. There are substantial amino acid differences between the N-terminus of the MCH-R present in humans and that present in other species such as mice. Such differences could result in, for example, the mouse MCH-R having different intrinsic properties and responsiveness to agonists and/or antagonists than the human MCH-R. The presence of a human MCH-R binding region provides for a "humanized" MCH-R chimeric receptor.

The transmembrane and intracellular domains characteristic of a non-human MCH-R can be used in conjunction with a non-human host to provide a more naturally occurring environment for these regions. For example, an MCH-R chimeric having mouse transmembrane and intracellular domains are preferably used in murine cells lines or in transgenic mice.

MCH-R chimeric proteins may contain regions other than extracellular, transmembrane, and intracellular domains that do not substantially decrease the activity of the protein. Preferably, additional regions do not cause a decrease of more than about 25% of MCH-R activity as measured using one or more of the assays

described in the examples provided below. Examples of additional regions that may be present include fluorescent protein regions and linker regions.

In an embodiment of the present invention, the MCH-R chimeric protein comprises: (a) an MCH binding region characteristic of a first species and (b) a transmembrane and intracellular domain region characteristic of a second species joined directly, or though a linker, to the carboxy side of the MCH binding region. Preferably, the protein comprises, consists, or consists essentially of an MCH-R polypeptide having a sequence similarity of at least about 75%, at least 85%, or at least 95% with either SEQ. ID. NO. 4 (human short form/mouse species chimeric MCH1R) or SEQ. ID. NO. 5 (human long form/mouse species chimeric). Even more preferably, the protein comprises, consists essentially of, or consists of, SEQ. ID. NO. 4 or SEQ. ID. NO. 5.

Sequence similarity for polypeptides can be determined by BLAST. (Altschul *et al.*, 1997. *Nucleic Acids Res. 25*, 3389-3402, hereby incorporated by reference herein.) In an embodiment of the present invention, sequence similarity is determined using tBLASTn search program with the following parameters: MATRIX:BLOSUM62, PER RESIDUE GAP COST: 11, and Lambda ratio: 1.

Differences in naturally occurring amino acids are due to different R groups. An R group effects different properties of the amino acid such as physical size, charge, and hydrophobicity. Amino acids can be divided into different groups as follows: neutral and hydrophobic (alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, and methionine); neutral and polar (glycine, serine, threonine, tyrosine, cysteine, asparagine, and glutamine); basic (lysine, arginine, and histidine); and acidic (aspartic acid and glutamic acid).

Generally, in substituting different amino acids it is preferable to exchange amino acids having similar properties. Substituting different amino acids within a particular group, such as substituting valine for leucine, arginine for lysine, and asparagine for glutamine are good candidates for not causing a change in polypeptide functioning.

Changes outside of different amino acids groups can also be made. Preferably, such changes are made taking into account the position of the amino acid to be substituted in the polypeptide. For example, arginine can substitute more freely for nonpolor amino acids in the interior of a polypeptide then glutamate because of its long aliphatic side chain. (*See*, Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, Supplement 33 Appendix 1C.)

MCH-R Fusion Proteins

MCH-R fusion proteins contain an MCH-R polypeptide region and a fluorescent protein region either directly joined together or joined together through a linker. These regions provide MCH-R activity and a marker for evaluating MCH-R dynamics.

An MCH-R polypeptide region provides functional MCH-R activity and includes naturally occurring MCH-R, chimeric MCH-R, and derivatives thereof. Preferred derivatives thereof have a sequence similarity of at least about 75%, at least about 85%, or at least about 95% to a naturally occurring MCH-R or a chimeric MCH-R described herein.

A fluorescent protein region contains a chromophore that fluoresces. Preferably, the fluorescent protein region is the green fluorescent protein of the jellyfish *Aequorea victoria* or a derivative thereof. Preferred derivatives have a sequence similarity of at least about 75%, at least about 85%, or at least about 95% to the *Aequorea victoria* green fluorescent protein (GFP). The *Aequorea victoria* green fluorescent protein and examples of derivatives thereof are described by Cormack *et al.*, 1996. *Gene 17*, 33-38; Yang *et al.*, 1996. *Nucleic Acids Research 24*, 4592-4593; Tsien *et al.*, U.S. Patent No. 5,625,048; Tsien *et al.*, U.S. Patent No. 5,777,079; and Cormack *et al.*, U.S. Patent No. 5,804,387 (each of which are hereby incorporated by reference herein).

In different embodiments the MCH-R polypeptide region comprises, consists essentially of, or consists of, a sequence selected from the group consisting of: SEQ. ID. NO. 1, SEQ. ID. NO. 2, SEQ. ID. NO. 3, SEQ. ID. NO. 4, and SEQ. ID. NO. 5; and the fluorescent polypeptide region comprises, consists essentially of, or consists of, an amino acid sequence selected from the group consisting of SEQ. ID. NO. 6 (GFP), SEQ. ID. NO. 7 (EGFP), SEQ. ID. NO. 8 (Emerald), SEQ. ID. NO. 9 (Topaz), and SEQ. ID. NO. 10 (W1b). EGFP, Emerald, Topaz, and W1b are derivatives of GFP.

The optionally present linker is a polypeptide region that is preferably from 1 to about 100 amino acids in length. In different embodiments the linker is up to 75, 50 or 25 amino acids in length.

Preferably, the MCH-R fusion protein comprises, consists essentially of, or consists of, the MCH-R polypeptide region and the fluorescent polypeptide region. More preferably, the protein comprises, consists essentially of, or consists of,

an amino acid sequence selected from the group consisting of: SEQ. ID. NO. 11 (mouse MCH1R-linker-EGFP), SEQ. ID. NO. 12 (mouse MCH1R/EGFP direct fusion), SEQ. ID. NO. 13 (human short form/mouse species chimeric MCH1R-linker-EGFP), or SEQ. ID. NO. 14 (human long form/mouse species chimeric MCH1R-linker-EGFP).

MCH-R Chimeric and Fusion Proteins Nucleic Acid and Expression

MCH-R chimeric and fusion proteins can be produced using techniques well known in the art. Preferably, such proteins are produced by recombinant expression inside a host cell by way of an expression vector or by way of nucleic acid integrated into the host genome. Examples of nucleic acid sequences encoding for MCH-R polypeptide regions, fluorescent protein regions, MCH-R chimeric proteins, and MCH-R fusion proteins are provided for by SEQ. ID. NOs. 15-29 (see Example 1, *infra*).

Starting with a particular amino acid sequence and the known degeneracy of the genetic code, a large number of different encoding nucleic acid sequences can be obtained. The degeneracy of the genetic code arises because almost all amino acids are encoded for by different combinations of nucleotide triplets or codons. The translation of a particular codon into a particular amino acid is well known in the art (*see, e.g.*, Lewin *GENES IV*, p. 119, Oxford University Press, 1990). Amino acids are encoded for by codons as follows:

A=Ala=Alanine: codons GCA, GCC, GCG, GCU
C=Cys=Cysteine: codons UGC, UGU
D=Asp=Aspartic acid: codons GAC, GAU
E=Glu=Glutamic acid: codons GAA, GAG
F=Phe=Phenylalanine: codons UUC, UUU
G=Gly=Glycine: codons GGA, GGC, GGG, GGU
H=His=Histidine: codons CAC, CAU
I=Ile=Isoleucine: codons AUA, AUC, AUU
K=Lys=Lysine: codons AAA, AAG
L=Leu=Leucine: codons UUA, UUG, CUA, CUC, CUG, CUU
M=Met=Methionine: codon AUG
N=Asn=Asparagine: codons AAC, AAU
P=Pro=Proline: codons CCA, CCC, CCG, CCU
Q=Gln=Glutamine: codons CAA, CAG

R=Arg=Arginine: codons AGA, AGG, CGA, CGC, CGG, CGU
S=Ser=Serine: codons AGC, AGU, UCA, UCC, UCG, UCU
T=Thr=Threonine: codons ACA, ACC, ACG, ACU
V=Val=Valine: codons GUA, GUC, GUG, GUU
W=Trp=Tryptophan: codon UGG
Y=Tyr=Tyrosine: codons UAC, UAU

Examples of techniques for introducing nucleic acid into a cell and expressing the nucleic acid to produce protein are provided in references such as Ausubel, *Current Protocols in Molecular Biology,* John Wiley, 1987-1998, and Sambrook, *et al.*, in *Molecular Cloning, A Laboratory Manual,* 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

An expression vector contains recombinant nucleic acid encoding for a polypeptide along with regulatory elements for proper transcription and processing. The recombinant nucleic acid contains two or more nucleic acid regions not naturally associated with each other. Exogenous regulatory elements such as an exogenous promoter can be useful for expressing recombinant nucleic acid in a particular host. Examples of expression vectors are cloning vectors, modified cloning vectors, specifically designed plasmids, and viruses.

Generally, the regulatory elements that are present in an expression vector include a transcriptional promoter, a ribosome binding site, a terminator, and an optionally present operator. Another preferred element is a polyadenylation signal providing for processing in eukaryotic cells. Preferably, an expression vector also contains an origin of replication for autonomous replication in a host cell, a selectable marker, a limited number of useful restriction enzyme sites, and a potential for high copy number.

Expression vectors providing suitable levels of polypeptide expression in different hosts are well known in the art. Mammalian expression vectors well known in the art include pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), pCI-neo (Promega) and .lambda.ZD35 (ATCC 37565). Bacterial expression vectors well known in the art include pET11a (Novagen), lambda gt11 (Invitrogen), pcDNAII (Invitrogen), and pKK223-3 (Pharmacia). Fungal cell expression vectors well known

in the art include pYES2 (Invitrogen) and Pichia expression vector (Invitrogen). Insect cell expression vectors well known in the art include Blue Bac III (Invitrogen).

Recombinant host cells may be prokaryotic or eukaryotic. Examples of recombinant host cells include the following: bacteria such as *E. coli*; fungal cells such as yeast; mammalian cells such as human, bovine, porcine, monkey, hampster, and rodent; and insect cells such as Drosophila and silkworm derived cell lines. Commercially available mammalian cell lines include L cells L-M(TK.sup.-) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70),.COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

To enhance expression in a particular host it may be useful to modify the sequence to take into account codon usage of the host. Codon usage of different organisms are well known in the art. (See, Ausubel, *Current Protocols in Molecular Biology,* John Wiley, 1987-1998, Supplement 33 Appendix 1C.)

Expression vectors may be introduced into host cells using standard techniques. Examples of such techniques include transformation, transfection, lipofection, protoplast fusion, and electroporation.

Nucleic acid encoding for a polypeptide can be expressed in a cell without the use of an expression vector employing, for example, synthetic mRNA or native mRNA. Additionally, mRNA can be translated in various cell-free systems such as wheat germ extracts and reticulocyte extracts, as well as in cell based systems, such as frog oocytes. Introduction of mRNA into cell based systems can be achieved, for example, by microinjection.

Techniques for producing transgenic animals are well known in the art. Examples of such techniques are provided for by Teratocarcinomas and embryonic stem cells: a practical approach. Ed. By E. J. Robertson, IRL Press Limited, Oxford, England (1987); and Gene Targeting: a practical approach. Ed. By A. L. Joyner, Oxford University Press Inc. New York, NY (1993).

G-Protein Coupled Receptor Assays

MCH-R is G-protein coupled receptor. Techniques for measuring different G-protein activities, such as Gi/o, Gs, and Gq are well known in the art. MCH-R activity is preferably assayed for by measuring either Gi/o or Gq.

Gi/o and Gs activity can be measured using techniques such as a melonaphore assay, measuring cAMP production, measuring inhibition of cAMP accumulation, and measuring binding of ^{35}S-GTP. cAMP can be measured using different techniques such as radioimmunoassay and indirectly by cAMP responsive gene reporter proteins.

Gq activity can be measured using techniques such as those measuring intracellular Ca^{2+}. Examples of techniques well known in the art that can be employed to measure Ca^{2+} include the use of dyes such as Fura-2 and the use of Ca^{2+}-bioluminescent sensitive reporter proteins such as aequorin. An example of a cell line employing aequorin to measure G-protein activity is HEK293/aeq17. (Button *et al.*,1993. *Cell Calcium 14*, 663-671, and Feighner *et al.*, 1999. *Science 284*, 2184-2188, both of which are hereby incorporated by reference herein.)

Functional assays can be performed using individual compounds or preparations containing different compounds. A preparation containing different compounds where one or more compounds affect MCH-R chimeric or fusion protein activity can be divided into smaller groups of compounds to identify the compound(s) affecting MCH-R chimeric or fusion protein activity. In an embodiment of the present invention a test preparation containing at least 10 compounds is used in a functional assay.

Functional assays can be performed using recombinantly produced MCH-R chimeric or fusion protein present in different environments. Such environments include, for example, cell extracts and purified cell extracts containing the MCH-R chimeric or fusion protein expressed from recombinant nucleic acid and an appropriate membrane for the polypeptide; and the use of a purified MCH-R chimeric or fusion protein produced by recombinant means that is introduced into a different environment suitable for measuring G-protein activity.

Fluorescent Protein Assays

Fluorescent protein joined to an MCH receptor can be employed to study different aspects of receptor dynamics including receptor sequestration, receptor densitization, and receptor localization. The fluorescent protein can be used in *in vitro* or *in vivo* systems.

In vitro applications of fluorescent proteins can be performed using techniques well known in the art. Examples of such techniques are provided by Barak *et al.*, 1997. *Mol Pharm. 5*, 177-184; Tarasova *et al.*, 1997. *J. Biol. Chem. 272*,

14817-14824; Lin *et al.*, 1998. *Mol. Cell. Endo. 146*, 27-37; Tarasova *et al.*, 1998. *J. Biol. Chem. 273*, 15883-15886; Kallal *et al.*, 1998. *J. Biol. Chem. 273*, 322-328; Groake *et al.*, 1999. *J. Biol. Chem. 274*, 23263-23269; Doherty *et al.*, 1999. *Biochem. J. 341*, 415-422; Brock *et al.*, 1999. *Proc. Natl. Acad. Sci. USA 96*, 10123-10128; Cornea *et al.*, 1999. *Endocrinology 140*, 4272-4280; and Lembo *et al.*, 1999. *Nat. Cell Biol. 1*, 267-271 (these references are not admitted to be prior art to the claimed invention).

In vivo applications of fluorescent proteins can be performed using techniques well known in the art. Examples of such techniques are provided by Mombaerts *et al.*, 1996. *Cell 87*, 675-686; Rodriquez *et al.*, 1999. *Cell 97*, 199-208; Spergel *et al.*, 1999. *J. Neurosci. 1*, 2037-2050; and Zuo *et al.*, 1999. *Proc. Natl. Acad. Sci. USA 96*, 14100-14105 (these references are not admitted to be prior art to the claimed invention).

EXAMPLES

Examples are provided below to further illustrate different features and advantages of the present invention. The examples also illustrate useful methodology for practicing the invention. These examples do not limit the claimed invention.

Example 1:

Amino acid and nucleic acid sequence information for SEQ. ID. NOs. 1-29 are provided below. SEQ. ID. NOs. 1-29 include examples of polypeptide and encoding nucleic acid sequences for MCH-R polypeptide regions, fluorescent polypeptide regions, fusion proteins and chimeric proteins. In some cases the encoding nucleic acid is shown with additional nucleic acid upsteam or downstream from an open reading frame.

SEQ. ID. NO. 1: Human long form MCH1R

MSVGAMKKGVGRAVGLGGGSGCQATEEDPLPNCGACAPGQGGRRWRLPQP
AWVEGSSARLWEQATGTGWMDLEASLLPTGPNASNTSDGPDNLTSAGSPPR
TGSISYINIIMPSVFGTICLLGIIGNSTVIFAVVKKSKLHWCNNVPDIFIINLSVVD
LLFLLGMPFMIHQLMGNGVWHFGETMCTLITAMDANSQFTSTYILTAMAIDR
YLATVHPISSTKFRKPSVATLVICLLWALSFISITPVWLYARLIPFPGGAVGCGI
RLPNPDTDLYWFTLYQFFLAFALPFVVITAAYVRILQRMTSSVAPASQRSIRLR

TKRVTRTAIAICLVFFVCWAPYYVLQLTQLSISRPTLTFVYLYNAAISLGYANS
CLNPFVYIVLCETFRKRLVLSVKPAAQGQLRAVSNAQTADEERTESKGT

SEQ. ID. NO. 2: Human short form MCH1R

MDLEASLLPTGPNASNTSDGPDNLTSAGSPPRTGSISYINIIMPSVFGTICLLGIIG
NSTVIFAVVKKSKLHWCNNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGVWH
FGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISSTKFRKPSVATLVI
CLLWALSFISITPVWLYARLIPFPGGAVGCGIRLPNPDTDLYWFTLYQFFLAFA
LPFVVITAAYVRILQRMTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWAPY
YVLQLTQLSISRPTLTFVYLYNAAISLGYANSCLNPFVYIVLCETFRKRLVLSV
KPAAQGQLRAVSNAQTADEERTESKGT

SEQ. ID. NO. 3: Mouse MCH1R

MDLQASLLSTGPNASNISDGQDNFTLAGPPPRTRSVSYINIIMPSVFGTICLLGI
VGNSTVIFAVVKKSKLHWCSNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGV
WHFGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISSTKFRKPSMAT
LVICLLWALSFISITPVWLYARLIPFPGGAVGCGIRLPNPDTDLYWFTLYQFFLA
FALPFVVITAAYVKILQRMTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWA
PYYVLQLTQLSISRPTLTFVYLYNAAISLGYANSCLNPFVYIVLCETFRKRLVLS
VKPAAQGQLRTVSNAQTADEERTESKGT

SEQ. ID. NO. 4: Human short form/mouse species chimeric MCH1R

MDLEASLLPTGPNASNTSDGPDNLTSAGSPPRTGSISYINIIMPSVFGTICLLGIIG
NSTVIFAVVKKSKLHWCNNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGVWH
FGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISSTKFRKPSMATLVI
CLLWALSFISITPVWLYARLIPFPGGAVGCGIRLPNPDTDLYWFTLYQFFLAFA
LPFVVITAAYVKILQRMTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWAPY
YVLQLTQLSISRPTLTFVYLYNAAISLGYANSCLNPFVYIVLCETFRKRLVLSV
KPAAQGQLRTVSNAQTADEERTESKGT

SEQ. ID. NO. 5: Human long form/mouse species chimeric MCH1R

MSVGAMKKGVGRAVGLGGGSGCQATEEDPLPNCGACAPGQGGRRWRLPQP
AWVEGSSARLWEQATGTGWMDLEASLLPTGPNASNTSDGPDNLTSAGSPPR
TGSISYINIIMPSVFGTICLLGIIGNSTVIFAVVKKSKLHWCNNVPDIFIINLSVVD
LLFLLGMPFMIHQLMGNGVWHFGETMCTLITAMDANSQFTSTYILTAMAIDR

YLATVHPISSTKFRKPSMATLVICLLWALSFISITPVWLYARLIPFPGGAVGCGI
RLPNPDTDLYWFTLYQFFLAFALPFVVITAAYVKILQRMTSSVAPASQRSIRLR
TKRVTRTAIAICLVFFVCWAPYYVLQLTQLSISRPTLTFVYLYNAAISLGYANS
CLNPFVYIVLCETFRKRLVLSVKPAAQGQLRTVSNAQTADEERTESKGT

SEQ. ID. NO. 6: GFP

MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKL
PVPWPTLVTTFSYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGN
YKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQ
KNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKD
PNEKRDHMVLLEFVTAAGITHGMDELYK

SEQ. ID. NO. 7: EGFP

MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGK
LPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDG
NYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADK
QKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSK
DPNEKRDHMVLLEFVTAAGITLGMDELYK

SEQ. ID. NO. 8: Emerald

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr
Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr
Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr
Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly
His Lys Leu Glu Tyr Asn Tyr Asn Ser His Lys Val Tyr Ile Thr Ala Asp Lys Gln Lys
Asn Gly Ile Lys Val Asn Phe Lys Thr Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu
Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn
His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met
Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys

SEQ. ID. NO. 9: Topaz

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr
Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr
Leu Val Thr Thr Phe Gly Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Arg
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr
Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly
His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys
Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu
Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn
His Tyr Leu Ser Tyr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met
Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys

SEQ. ID. NO. 10: W1B

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp
Gly Asp Val Asn Gly His Arg Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr
Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr
Leu Val Thr Thr Leu Thr Trp Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr
Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly
His Lys Leu Glu Tyr Asn Tyr Ile Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys
Asn Gly Ile Lys Ala His Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu
Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn
His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met
Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys

SEQ. ID. NO. 11: Mouse MCH1R-linker-EGFP

MDLQASLLSTGPNASNISDGQDNFTLAGPPPRTRSVSYINIIMPSVFGTICLLGI
VGNSTVIFAVVKKSKLHWCSNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGV
WHFGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISSTKFRKPSMAT
LVICLLWALSFISITPVWLYARLIPFPGGAVGCGIRLPNPDTDLYWFTLYQFFLA
FALPFVVITAAYVKILQRMTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWA
PYYVLQLTQLSISRPTLTFVYLYNAAISLGYANSCLNPFVYIVLCETFRKRLVLS

VKPAAQGQLRTVSNAQTADEERTESKGTVDGTAGPGSIATMVSKGEELFTGV
VPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTL
TYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFE
GDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIR
HNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVL
LEFVTAAGITLGMDELYK

SEQ. ID. NO. 12: Mouse MCH1R/EGFP direct fusion

MDLQASLLSTGPNASNISDGQDNFTLAGPPPRTRSVSYINIIMPSVFGTICLLGI
VGNSTVIFAVVKKSKLHWCSNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGV
WHFGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISSTKFRKPSMAT
LVICLLWALSFISITPVWLYARLIPFPGGAVGCGIRLPNPDTDLYWFTLYQFFLA
FALPFVVITAAYVKILQRMTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWA
PYYVLQLTQLSISRPTLTFVYLYNAAISLGYANSCLNPFVYIVLCETFRKRLVLS
VKPAAQGQLRTVSNAQTADEERTESKGTMVSKGEELFTGVVPILVELDGDVN
GHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPD
HMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGI
DFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLAD
HYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLG
MDELYK

SEQ. ID. NO. 13: Human short form/mouse species chimeric MCH1R-linker-EGFP

MDLEASLLPTGPNASNTSDGPDNLTSAGSPPRTGSISYINIIMPSVFGTICLLGIIG
NSTVIFAVVKKSKLHWCNNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGVWH
FGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISSTKFRKPSMATLVI
CLLWALSFISITPVWLYARLIPFPGGAVGCGIRLPNPDTDLYWFTLYQFFLAFA
LPFVVITAAYVKILQRMTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWAPY
YVLQLTQLSISRPTLTFVYLYNAAISLGYANSCLNPFVYIVLCETFRKRLVLSV
KPAAQGQLRTVSNAQTADEERTESKGTVDGTAGPGSIATMVSKGEELFTGVV
PILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLT
YGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEG
DTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRH
NIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLL
EFVTAAGITLGMDELYK

SEQ. ID. NO. 14: Human long form/mouse species chimeric MCH1R-linker-EGFP

MSVGAMKKGVGRAVGLGGGSGCQATEEDPLPNCGACAPGQGGRRWRLPQP
AWVEGSSARLWEQATGTGWMDLEASLLPTGPNASNTSDGPDNLTSAGSPPR
TGSISYINIIMPSVFGTICLLGIIGNSTVIFAVVKKSKLHWCNNVPDIFIINLSVVD
LLFLLGMPFMIHQLMGNGVWHFGETMCTLITAMDANSQFTSTYILTAMAIDR
YLATVHPISSTKFRKPSMATLVICLLWALSFISITPVWLYARLIPFPGGAVGCGI
RLPNPDTDLYWFTLYQFFLAFALPFVVITAAYVKILQRMTSSVAPASQRSIRLR
TKRVTRTAIAICLVFFVCWAPYYVLQLTQLSISRPTLTFVYLYNAAISLGYANS
CLNPFVYIVLCETFRKRLVLSVKPAAQGQLRTVSNAQTADEERTESKGTVDGT
AGPGSIATMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLT
LKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQE
RTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSH
NVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHY
LSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK

SEQ. ID. NO. 15: Human long form MCH1R cDNA

ATGTCAGTGGGAGCCATGAAGAAGGGAGTGGGGAGGGCAGTTGGGCTTG
GAGGCGGCAGCGGCTGCCAGGCTACGGAGGAAGACCCCCTTCCCAACTGC
GGGGCTTGCGCTCCGGGACAAGGTGGCAGGCGCTGGAGGCTGCCGCAGC
CTGCGTGGGTGGAGGGGAGCTCAGCTCGGTTGTGGGAGCAGGCGACCGG
CACTGGCTGGATGGACCTGGAAGCCTCGCTGCTGCCCACTGGTCCCAACG
CCAGCAACACCTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCT
CCTCGCACGGGGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTC
GGCACCATCTGCCTCCTGGGCATCATCGGGAACTCCACGGTCATCTTCGCG
GTCGTGAAGAAGTCCAAGCTGCACTGGTGCAACAACGTCCCCGACATCTT
CATCATCAACCTCTCGGTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTT
CATGATCCACCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCA
TGTGCACCCTCATCACGGCCATGGATGCCAATAGTCAGTTCACCAGCACC
TACATCCTGACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCC
ATCTCTTCCACGAAGTTCCGGAAGCCCTCTGTGGCCACCCTGGTGATCTGC
CTCCTGTGGGCCCTCTCCTTCATCAGCATCACCCCTGTGTGGCTGTATGCC
AGACTCATCCCCTTCCCAGGAGGTGCAGTGGGCTGCGGCATACGCCTGCC
CAACCCAGACACTGACCTCTACTGGTTCACCCTGTACCAGTTTTTCCTGGC

CTTTGCCCTGCCTTTTGTGGTCATCACAGCCGCATACGTGAGGATCCTGCA
GCGCATGACGTCCTCAGTGGCCCCCGCCTCCCAGCGCAGCATCCGGCTGC
GGACAAAGAGGGTGACCCGCACAGCCATCGCCATCTGTCTGGTCTTCTTT
GTGTGCTGGGCACCCTACTATGTGCTACAGCTGACCCAGTTGTCCATCAGC
CGCCCGACCCTCACCTTTGTCTACTTATACAATGCGGCCATCAGCTTGGGC
TATGCCAACAGCTGCCTCAACCCCTTTGTGTACATCGTGCTCTGTGAGACG
TTCCGCAAACGCTTGGTCCTGTCGGTGAAGCCTGCAGCCCAGGGGCAGCT
TCGCGCTGTCAGCAACGCTCAGACGGCTGACGAGGAGAGGACAGAAAGC
AAAGGCACCTGA

SEQ. ID. NO. 16: Human short form MCH1R cDNA

ATGGACCTGGAAGCCTCGCTGCTGCCCACTGGTCCCAATGCCAGCAACAC
CTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCTCCTCGCACGG
GGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTCGGCACCATCT
GCCTCCTGGGCATCATCGGGAACTCCACGGTCATCTTCGCGGTCGTGAAG
AAGTCCAAGCTGCACTGGTGCAACAACGTCCCCGACATCTTCATCATCAA
CCTCTCGGTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTTCATGATCCA
CCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCATGTGCACCC
TCATCACGGCCATGGATGCCAATAGTCAGTTCACCAGCACCTACATCCTG
ACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCCATCTCTTCC
ACGAAGTTCCGGAAGCCCTCTGTGGCCACCCTGGTGATCTGCCTCCTGTGG
GCCCTCTCCTTCATCAGCATCACCCCTGTGTGGCTGTATGCCAGACTCATC
CCCTTCCCAGGAGGTGCAGTGGGCTGCGGCATACGCCTGCCCAACCCAGA
CACTGACCTCTACTGGTTCACCCTGTACCAGTTTTTCCTGGCCTTTGCCCTG
CCTTTTGTGGTCATCACAGCCGCATACGTGAGGATCCTGCAGCGCATGAC
GTCCTCAGTGGCCCCCGCCTCCCAGCGCAGCATCCGGCTGCGGACAAAGA
GGGTGACCCGCACAGCCATCGCCATCTGTCTGGTCTTCTTTGTGTGCTGGG
CACCCTACTATGTGCTACAGCTGACCCAGTTGTCCATCAGCCGCCCGACCC
TCACCTTTGTCTACTTATACAATGCGGCCATCAGCTTGGGCTATGCCAACA
GCTGCCTCAACCCCTTTGTGTACATCGTGCTCTGTGAGACGTTCCGCAAAC
GCTTGGTCCTGTCGGTGAAGCCTGCAGCCCAGGGGCAGCTTCGCGCTGTC
AGCAACGCTCAGACGGCTGACGAGGAGAGGACAGAAAGCAAAGGCACCT
GA

SEQ. ID. NO. 17: Mouse MCH1R cDNA

Nucleic acid sequence start and stop codons are highlighted:

GGCGGTAGAGGAAGACCCTTTTCTGGACTGCGGGGCTCAAGCTCCGGACA
AGGCGGTGGAGGGCGCTGGAGGCTGCCGCAGCCTGCGTGGGTGGACGGG
CGCTCCACTCCAGGGAGCAGGCGACCTGCACCGGCTGCATGGATCTGCAA
GCCTCGTTGCTGTCCACTGGCCCCAATGCCAGCAACATCTCCGATGGCCA
GGATAATTTCACATTGGCGGGGCCACCTCCTCGCACAAGGAGTGTCTCCT
ACATCAACATCATCATGCCTTCAGTGTTTGGTACCATCTGTCTCCTGGGCA
TTGTGGGAAACTCCACAGTCATTTTTGCCGTGGTGAAGAAATCCAAGCTG
CACTGGTGCAGCAACGTCCCTGACATCTTCATCATCAACCTCTCTGTGGTG
GATCTGCTTTTCCTGCTGGGCATGCCTTTCATGATCCACCAGCTCATGGGT
AATGGTGTCTGGCACTTTGGGGAAACCATGTGCACCCTCATCACAGCCAT
GGACGCCAACAGTCAGTTCACCAGCACCTACATCCTGACTGCTATGGCCA
TTGACCGCTACTTGGCCACCGTCCATCCCATCTCCTCCACCAAGTTCCGGA
AGCCCTCCATGGCCACCCTGGTGATCTGCCTCCTGTGGGCTCTCTCGTTCA
TTAGCATCACTCCTGTGTGGCTCTATGCCAGGCTTATCCCCTTCCCAGGGG
GTGCTGTGGGCTGTGGCATCCGCCTACCAAACCCAGATACTGATCTTTACT
GGTTCACTCTGTATCAGTTTTTCCTGGCCTTCGCCCTTCCGTTTGTGGTCAT
CACTGCTGCGTACGTGAAAATACTACAGCGCATGACGTCTTCGGTGGCCC
CAGCCTCTCAACGCAGCATCCGGCTTCGGACAAAGAGGGTGACCCGCACA
GCCATTGCCATCTGTCTGGTCTTCTTTGTGTGCTGGGCGCCCTACTACGTG
CTGCAGCTGACCCAGTTGTCCATCAGCCGCCCGACCCTCACATTCGTCTAC
CTGTACAATGCGGCCATCAGCTTGGGCTATGCCAACAGCTGCCTCAATCC
CTTTGTGTACATAGTACTCTGTGAGACCTTTCGAAAACGCTTGGTGCTGTC
GGTGAAGCCCGCGGCCCAGGGGCAGCTTCGCACGGTCAGCAATGCTCAGA
CAGCTGACGAGGAGAGGACAGAAAGCAAAGGCACCTGACAATCCCCCCC
GGTCACCTCCAAGTCAGGTCACCGCATCAAACCATGGGGAGAGATACTGA
GATAAACCCGGGGCTACCCTGGGAGGATGCAGAAGCTGGAGGCTGGGGG
CTTGTAGCAAACCACATTCCACGGGGCCCACAAATTGCTAGGGAGGCTTG
CAGCCTGGTTTGGGGGGGAAGCCTCAGACTGCAGGGATCCCCTTGACAGA
ATAGAAGCGGAGCAAGAAGGAAAGGGTGGTTTGACTGGTTCTCGGGGTCT
GTATCTGTTGGCTCGCATATATCTTTCTCTCAAGGGAAGAAGGCGGAGGT
GCCTAGCTGGGTTCCTTTAAAACTAGGCAGGGCTAGGATCTGAGCAGCTA
GGGCTCTACTGTGAGACTGGGCAAGCCGAGCGTTCCCTCCCATCTCTCATT
GGTGTTGATAGAAGGCAGTCTTTCTCCCAAGCTGGTGGATCTCCTGAAGC

ACGCTGCCTGGGCTCCAGCATCCTGTGCGGATTTCACGTTCTCTTTAGGGG
ATGCATGTTGACACTGGGGTGTGGGCTCTGAGCCCACAGGAGTTTAAAAA
ACCAAAAGAGCTCAGAGTGTCGAGAGAGACCCAATCACCGAGAATGACA
AGGCAACCTGGGGTGGATGTGGATCTTGAAACTAATAAAAAGGGGTTTTC
ACAGTGACAGCGACATTCTCTTCATAGGGCACAGCTGTCAGTCTATGGCT
GATCCAGAGCGAGCATCCATGAATTCTGCATGTGCAGGGGTCACTCTAAT
ACCTGATATGTTGGCATCATCTTTGTGCTTGAGCCTTCCNCTCCCAAATGG
GAATGAAATAAAGGCAAATTCCCNCCCCCCCCAAAAAAGGGGNAAAAAA
AAAAAAAAAAAAAAAAAAAA

SEQ. ID. NO. 18: Mouse MCH1R genomic DNA

Nucleic acid sequence start and stop codons, as well as intron borders, are highlighted:

GGCGGTAGAGGAAGACCCTTTTCTGGACTGCGGGGCTCAAGCTCCGGACA
AGGCGGTGGAGGGCGCTGGAGGCTGCCGCAGCCTGCGTGGGTGGACGGG
CGCTCCACTCCAGGGAGCAGGCGACCTGCACCGGCTGC**ATG**GATCTGCAA
GCCTCGTTGCTGTCCACTGGCCCCAATGCCAGCAACATCTCCGATGGCCA
GGATAATTTCACATTGGCGGGTGAGTCGAGTTGGAGTCCTCCCTCCTCCG
GGATGGGTGTGGAAAATGGGAAGGTTTCACCTCCCAAGCCAAACTGCCTG
GGAAACTTTATCTTACAGTTCTTGGTGATAAGATCTGCAGTCGGCTTTGCC
TGAAGAGGAAGAGGAGAGGAGGGGACACCAGCTAGGACAGAAGGGGCA
GGGAGGAATAGAGATGGGGCAGAGGCACATTTAGAAACAACAAGGGTTG
GTGACAAGACGTGAGGCAGGCTTGAGGGGAAAGCTTGCTGATGAGTCCCA
AATATGCTTTGCAGGGGGGGGGGGGGGGGAATCAAGGCTGGAGAAGCAA
GCAAGCAAGACAGCAAGACAGCGGGCGGGTAGTATGTGGGAGCCAGCAG
AAGCGCTTTGATTCACCGCTATCCTGGGCTCAATCCTCTGGCCTCGCACTG
GGGAAATGGGGTCTGAGTGGTCCTTGCTGTCTTCTGGCAAAGGCTGCTGG
GAGCAAAAGACTTCACAGGGCGTGAGAGGATTAACTTTTCTGGTGAATTA
AGCTTCTTGACATTTGCAGAACGTCAATGCCTTAAAATTCTAGCTCTGAAG
GAGAAGGGAATGAAGGGGAAAGAGGGAAGGTTGGTGTGGAGAAATTCCC
AAGCTTCTGGGGTGTAACACAGCTCCAGTCCCTACCCTATTGGGAAAGCC
CAGACTCAGGAGACATGGTCCAAGGAAATCCCTGACAGAAAACCGGGAG
AGGGCAGGGCTGTGGAGCCTGAAACACACCCCACACCCATGGTGACAGTC
ACTTCTCACATATGCCTAGGAACCTATCTGAAACCTTTGGCCATCTCTCTC
TGAAAAGATGAGGCTGCAAATACACACACACACACACACACACACACAC

ACACACACACACACACACACACACACACACACACAAATGTCCTTCAAGCC
TTTTTGACAAGGTTTTCTGGTGGATCCCGGGGATATGAAGTTGTTCTCAGC
AGATATCTGGGAGTCTTGACTCCTGGCCCTCTGAGTAAATGGATGAAGCG
AAGAAGAATGGGGTCCTCTGAGTAACAGGTGGATCTAGAAAATCCTATAG
GAGTCACCAGGGCACGGTGGAGGAGGGTAAGGTACAGAACTAACAATAG
CCCGAGAAGGGGAAACAGCAGGAGATGATTCCAGAGACGTAGTGACCCC
AAGCTGCAAGGGAAAGCATGAGGGGCCAGCAGGAAGGCCGACATGGCAG
GTTGTCAGCTTCTAGATCGGAAGGCGGGTCACACTTGCTCTTTCTATCCTC
AGGGCCACCTCCTCGCACAAGGAGTGTCTCCTACATCAACATCATCATGC
CTTCAGTGTTTGGTACCATCTGTCTCCTGGGCATTGTGGGAAACTCCACAG
TCATTTTTGCCGTGGTGAAGAAATCCAAGCTGCACTGGTGCAGCAACGTC
CCTGACATCTTCATCATCAACCTCTCTGTGGTGGATCTGCTTTTCCTGCTGG
GCATGCCTTTCATGATCCACCAGCTCATGGGTAATGGTGTCTGGCACTTTG
GGGAAACCATGTGCACCCTCATCACAGCCATGGACGCCAACAGTCAGTTC
ACCAGCACCTACATCCTGACTGCTATGGCCATTGACCGCTACTTGGCCACC
GTCCATCCCATCTCCTCCACCAAGTTCCGGAAGCCCTCCATGGCCACCCTG
GTGATCTGCCTCCTGTGGGCTCTCTCGTTCATTAGCATCACTCCTGTGTGG
CTCTATGCCAGGCTTATCCCCTTCCCAGGGGGTGCTGTGGGCTGTGGCATC
CGCCTACCAAACCCAGATACTGATCTTTACTGGTTCACTCTGTATCAGTTT
TTCCTGGCCTTCGCCCTTCCGTTTGTGGTCATCACTGCTGCGTACGTGAAA
ATACTACAGCGCATGACGTCTTCGGTGGCCCCAGCCTCTCAACGCAGCAT
CCGGCTTCGGACAAAGAGGGTGACCCGCACAGCCATTGCCATCTGTCTGG
TCTTCTTTGTGTGCTGGGCGCCCTACTACGTGCTGCAGCTGACCCAGTTGT
CCATCAGCCGCCCGACCCTCACATTCGTCTACCTGTACAATGCGGCCATCA
GCTTGGGCTATGCCAACAGCTGCCTCAATCCCTTTGTGTACATAGTACTCT
GTGAGACCTTTCGAAAACGCTTGGTGCTGTCGGTGAAGCCCGCGGCCCAG
GGGCAGCTTCGCACGGTCAGCAATGCTCAGACAGCTGACGAGGAGAGGA
CAGAAAGCAAAGGCACCTGACAATCCCCCCCGGTCACCTCCAAGTCAGGT
CACCGCATCAAACCATGGGGAGAGATACTGAGATAAACCCGGGGCTACC
CTGGGAGGATGCAGAAGCTGGAGGCTGGGGGCTTGTAGCAAACCACATTC
CACGGGGCCCACAAATTGCTAGGGAGGCTTGCAGCCTGGTTTGGGGGGGA
AGCCTCAGACTGCAGGGATCCCCTTGACAGAATAGAAGCGGAGCAAGAA
GGAAAGGGTGGTTTGACTGGTTCTCGGGGTCTGTATCTGTTGGCTCGCATA
TATCTTTCTCTCAAGGGAAGAAGGCGGAGGTGCCTAGCTGGGTTCCTTTA
AAACTAGGCAGGGCTAGGATCTGAGCAGCTAGGGCTCTACTGTGAGACTG

GGCAAGCCGAGCGTTCCCTCCCATCTCTCATTGGTGTTGATAGAAGGCAG
TCTTTCTCCCAAGCTGGTGGATCTCCTGAAGCACGCTGCCTGGGCTCCAGC
ATCCTGTGCGGATTTCACGTTCTCTTTAGGGGATGCATGTTGACACTGGGG
TGTGGGCTCTGAGCCCACAGGAGTTTAAAAAACCAAAAGAGCTCAGAGTG
TCGAGAGAGACCCAATCACCGAGAATGACAAGGCAACCTGGGGTGGATG
TGGATCTTGAAACTAATAAAAAGGGGTTTTCACAGTGACAGCGACATTCT
CTTCATAGGGCACAGCTGTCAGTCTATGGCTGATCCAGAGCGAGCATCCA
TGAATTCTGCATGTGCAGGGGTCACTCTAATACCTGATATGTTGGCATCAT
CTTTGTGCTTGAGCCTTCCNCTCCCAAATGGGAATGAAATAAAGGCAAAT
TCCCNCCCCCCCCCAAAAAAGGGGNAAAAAAAAAAAAAAAAAAAAAAAA
AA

SEQ. ID. NO. 19: Human short form/mouse species chimeric MCH1R

ATGGACCTGGAAGCCTCGCTGCTGCCCACTGGTCCCAATGCCAGCAACAC
CTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCTCCTCGCACGG
GGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTCGGCACCATCT
GCCTCCTGGGCATCATCGGGAACTCCACGGTCATCTTCGCGGTCGTGAAG
AAGTCCAAGCTGCACTGGTGCAACAACGTCCCCGACATCTTCATCATCAA
CCTCTCGGTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTTCATGATCCA
CCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCATGTGCACCC
TCATCACGGCCATGGATGCCAATAGTCAGTTCACCAGCACCTACATCCTG
ACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCCATCTCTTCC
ACGAAGTTCCGGAAGCCCTCCATGGCCACCCTGGTGATCTGCCTCCTGTG
GGCTCTCTCGTTCATTAGCATCACTCCTGTGTGGCTCTATGCCAGGCTTAT
CCCCTTCCCAGGGGGTGCTGTGGGCTGTGGCATCCGCCTACCAAACCCAG
ATACTGATCTTTACTGGTTCACTCTGTATCAGTTTTTCCTGGCCTTCGCCCT
TCCGTTTGTGGTCATCACTGCTGCGTACGTGAAAATACTACAGCGCATGAC
GTCTTCGGTGGCCCCAGCCTCTCAACGCAGCATCCGGCTTCGGACAAAGA
GGGTGACCCGCACAGCCATTGCCATCTGTCTGGTCTTCTTTGTGTGCTGGG
CGCCCTACTACGTGCTGCAGCTGACCCAGTTGTCCATCAGCCGCCCGACC
CTCACATTCGTCTACCTGTACAATGCGGCCATCAGCTTGGGCTATGCCAAC
AGCTGCCTCAATCCCTTTGTGTACATAGTACTCTGTGAGACCTTTCGAAAA
CGCTTGGTGCTGTCGGTGAAGCCCGCGGCCCAGGGGCAGCTTCGCACGGT
CAGCAATGCTCAGACAGCTGACGAGGAGAGGACAGAAAGCAAAGGCACC
TGA

SEQ. ID. NO. 20: Human long form/mouse species chimeric MCH1R

ATGTCAGTGGGAGCCATGAAGAAGGGAGTGGGGAGGGCAGTTGGGCTTG
GAGGCGGCAGCGGCTGCCAGGCTACGGAGGAAGACCCCCTTCCCAACTGC
GGGGCTTGCGCTCCGGGACAAGGTGGCAGGCGCTGGAGGCTGCCGCAGC
CTGCGTGGGTGGAGGGGAGCTCAGCTCGGTTGTGGGAGCAGGCGACCGG
CACTGGCTGGATGGACCTGGAAGCCTCGCTGCTGCCCACTGGTCCCAACG
CCAGCAACACCTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCT
CCTCGCACGGGGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTC
GGCACCATCTGCCTCCTGGGCATCATCGGGAACTCCACGGTCATCTTCGCG
GTCGTGAAGAAGTCCAAGCTGCACTGGTGCAACAACGTCCCCGACATCTT
CATCATCAACCTCTCGGTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTT
CATGATCCACCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCA
TGTGCACCCTCATCACGGCCATGGATGCCAATAGTCAGTTCACCAGCACC
TACATCCTGACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCC
ATCTCTTCCACGAAGTTCCGGAAGCCCTCCATGGCCACCCTGGTGATCTGC
CTCCTGTGGGCTCTCTCGTTCATTAGCATCACTCCTGTGTGGCTCTATGCC
AGGCTTATCCCCTTCCCAGGGGGTGCTGTGGGCTGTGGCATCCGCCTACCA
AACCCAGATACTGATCTTTACTGGTTCACTCTGTATCAGTTTTTCCTGGCCT
TCGCCCTTCCGTTTGTGGTCATCACTGCTGCGTACGTGAAAATACTACAGC
GCATGACGTCTTCGGTGGCCCCAGCCTCTCAACGCAGCATCCGGCTTCGG
ACAAAGAGGGTGACCCGCACAGCCATTGCCATCTGTCTGGTCTTCTTTGTG
TGCTGGGCGCCCTACTACGTGCTGCAGCTGACCCAGTTGTCCATCAGCCGC
CCGACCCTCACATTCGTCTACCTGTACAATGCGGCCATCAGCTTGGGCTAT
GCCAACAGCTGCCTCAATCCCTTTGTGTACATAGTACTCTGTGAGACCTTT
CGAAAACGCTTGGTGCTGTCGGTGAAGCCCGCGGCCCAGGGGCAGCTTCG
CACGGTCAGCAATGCTCAGACAGCTGACGAGGAGAGGACAGAAAGCAAA
GGCACCTGA

SEQ. ID. NO. 21: ***Aequorea victoria*** **Green Fluorescent Protein (GFP) cDNA**

Nucleic acid sequence start and stop codons are highlighted:

TACACACGAATAAAAGATAACAAAGATGAGTAAAGGAGAAGAACTTTTC
ACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCAC
AAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACT
TACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAAC

ACTTGTCACTACTTTCTCTTATGGTGTTCAATGCTTTTCAAGATACCCAGAT
CATATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGT
ACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTG
CTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAA
GGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATA
CAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATG
GAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTT
CAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGT
CCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGA
TCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTG
CTGGGATTACACATGGCATGGATGAACTATACAAATAAATGTCCAGACTT
CCAATTGACACTAAAGTGTCCGAACAATTACTAAAATCTCAGGGTTCCTG
GTTAAATTCAGGCTGAGATATTATTTATATATTTATAGATTCATTAAAATT
GTATGAATAATTTATTGATGTTATTGATAGAGGTTATTTTCTTATTAAACA
GGCTACTTGGAGTGTATTCTTAATTCTATATTAATTACAATTTGATTTGACT
TGCTCAAA

SEQ. ID. NO. 22: EGFP + Linker

Nucleic acid sequence start and stop codons are highlighted and a 12 amino acid linker sequence is denoted in lower case:

gtcgacggtaccgcgggcccgggatccatcgccacc**ATG**GTGAGCAAGGGCGAGGAGCTGTT
CACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCC
ACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAA
GCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGC
CCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTAC
CCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGG
CTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGA
CCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAG
CTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGC
TGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAG
AAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACG
GCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGAC
GGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT
GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCG

TGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGC
GGCCGC

SEQ. ID. NO. 23: Emerald

ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGT
CGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAG
GGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCAC
CACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTGACCT
ACGGCGTGCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGAC
TTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTC
TTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGG
GCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAG
GACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACA
AGGTCTATATCACCGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTC
AAGACCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACT
ACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAAC
CACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCG
CGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCG
GCATGGACGAGCTGTACAAGTAA

SEQ. ID. NO. 24: Topaz

ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGT
CGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAG
GGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCAC
CACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTCGGCT
ACGGCGTGCAGTGCTTCGCCCGCTACCCCGACCACATGCGCCAGCACGAC
TTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTC
TTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGG
GCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAG
GACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACA
ACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTC
AAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTA
CCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACC
ACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGC

GATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGG
CATGGACGAGCTGTACAAGTAA

SEQ. ID. NO. 25: W1B

ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGT
CGAGCTGGACGGCGACGTAAACGGCCACAGGTTCAGCGTGTCCGGCGAG
GGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCAC
CACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCT
GGGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGAC
TTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGTACCATCTTC
TTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGG
GCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAG
GACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACATCAGCCACA
ACGTCTATATCACCGCCGACAAGCAGAAGAACGGCATCAAGGCCCACTTC
AAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTA
CCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACC
ACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGC
GATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGG
CATGGACGAGCTGTACAAGTAA

SEQ. ID. NO. 26: Mouse MCH1R-linker-EGFP

Nucleic acid sequence start codon and start and stop codons for mouse MCH1R and EGFP, respectively, as well as intron borders, are highlighted and a 12 amino acid linker sequence is denoted in lower case:

ATGGATCTGCAAGCCTCGTTGCTGTCCACTGGCCCCAATGCCAGCAACAT
CTCCGATGGCCAGGATAATTTCACATTGGCG**GG**TGAGTCGAGTTGGAGTC
CTCCCTCCTCCGGGATGGGTGTGGAAAATGGGAAGGTTTCACCTCCCAAG
CCAAACTGCCTGGGAAACTTTATCTTACAGTTCTTGGTGATAAGATCTGCA
GTCGGCTTTGCCTGAAGAGGAAGAGGAGAGGAGGGGACACCAGCTAGGA
CAGAAGGGGCAGGGAGGAATAGAGATGGGGCAGAGGCACATTTAGAAAC
AACAAGGGTTGGTGACAAGACGTGAGGCAGGCTTGAGGGGAAAGCTTGC
TGATGAGTCCCAAATATGCTTTGCAGGGGGGGGGGGGGGGGAATCAAGG
CTGGAGAAGCAAGCAAGCAAGACAGCAAGACAGCGGGCGGGTAGTATGT
GGGAGCCAGCAGAAGCGCTTTGATTCACCGCTATCCTGGGCTCAATCCTC
TGGCCTCGCACTGGGGAAATGGGGTCTGAGTGGTCCTTGCTGTCTTCTGGC

AAAGGCTGCTGGGAGCAAAAGACTTCACAGGGCGTGAGAGGATTAACTTT
TCTGGTGAATTAAGCTTCTTGACATTTGCAGAACGTCAATGCCTTAAAATT
CTAGCTCTGAAGGAGAAGGGAATGAAGGGGAAAGAGGGAAGGTTGGTGT
GGAGAAATTCCCAAGCTTCTGGGGTGTAACACAGCTCCAGTCCCTACCCT
ATTGGGAAAGCCCAGACTCAGGAGACATGGTCCAAGGAAATCCCTGACA
GAAAACCGGGAGAGGGCAGGGCTGTGGAGCCTGAAACACACCCCACACC
CATGGTGACAGTCACTTCTCACATATGCCTAGGAACCTATCTGAAACCTTT
GGCCATCTCTCTCTGAAAAGATGAGGCTGCAAATACACACACACACACAC
ACACACACACACACACACACACACACACACACACACACACACACACACAAA
TGTCCTTCAAGCCTTTTTGACAAGGTTTTCTGGTGGATCCCGGGGATATGA
AGTTGTTCTCAGCAGATATCTGGGAGTCTTGACTCCTGGCCCTCTGAGTAA
ATGGATGAAGCGAAGAAGAATGGGGTCCTCTGAGTAACAGGTGGATCTA
GAAAATCCTATAGGAGTCACCAGGGCACGGTGGAGGAGGGTAAGGTACA
GAACTAACAATAGCCCGAGAAGGGGAAACAGCAGGAGATGATTCCAGAG
ACGTAGTGACCCCAAGCTGCAAGGGAAAGCATGAGGGGCCAGCAGGAAG
GCCGACATGGCAGGTTGTCAGCTTCTAGATCGGAAGGCGGGTCACACTTG
CTCTTTCTATCCTCAGGGCCACCTCCTCGCACAAGGAGTGTCTCCTACATC
AACATCATCATGCCTTCAGTGTTTGGTACCATCTGTCTCCTGGGCATTGTG
GGAAACTCCACAGTCATTTTTGCCGTGGTGAAGAAATCCAAGCTGCACTG
GTGCAGCAACGTCCCTGACATCTTCATCATCAACCTCTCTGTGGTGGATCT
GCTTTTCCTGCTGGGCATGCCTTTCATGATCCACCAGCTCATGGGTAATGG
TGTCTGGCACTTTGGGGAAACCATGTGCACCCTCATCACAGCCATGGACG
CCAACAGTCAGTTCACCAGCACCTACATCCTGACTGCTATGGCCATTGACC
GCTACTTGGCCACCGTCCATCCCATCTCCTCCACCAAGTTCCGGAAGCCCT
CCATGGCCACCCTGGTGATCTGCCTCCTGTGGGCTCTCTCGTTCATTAGCA
TCACTCCTGTGTGGCTCTATGCCAGGCTTATCCCCTTCCCAGGGGGTGCTG
TGGGCTGTGGCATCCGCCTACCAAACCCAGATACTGATCTTTACTGGTTCA
CTCTGTATCAGTTTTTCCTGGCCTTCGCCCTTCCGTTTGTGGTCATCACTGC
TGCGTACGTGAAAATACTACAGCGCATGACGTCTTCGGTGGCCCCAGCCT
CTCAACGCAGCATCCGGCTTCGGACAAAGAGGGTGACCCGCACAGCCATT
GCCATCTGTCTGGTCTTCTTTGTGTGCTGGGCGCCCTACTACGTGCTGCAG
CTGACCCAGTTGTCCATCAGCCGCCCGACCCTCACATTCGTCTACCTGTAC
AATGCGGCCATCAGCTTGGGCTATGCCAACAGCTGCCTCAATCCCTTTGTG
TACATAGTACTCTGTGAGACCTTTCGAAAACGCTTGGTGCTGTCGGTGAA
GCCCGCGGCCCAGGGGCAGCTTCGCACGGTCAGCAATGCTCAGACAGCTG

ACGAGGAGAGGACAGAAAGCAAAGGCACCgtcgacggtaccgcgggcccgggatccatcg
ccaccATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCC
TGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGC
GAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTG
CACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGA
CCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCAC
GACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCAT
CTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCG
AGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAG
GAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCC
ACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAA
CTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACC
ACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGAC
AACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAA
GCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTC
TCGGCATGGACGAGCTGTACAAGTAA

SEQ. ID. NO. 27: Mouse MCH1R/EGFP direct fusion

Nucleic acid sequence start codon and start and stop codons for mouse MCH1R and EGFP, respectively, as well as intron borders, are highlighted:

ATGGATCTGCAAGCCTCGTTGCTGTCCACTGGCCCCAATGCCAGCAACAT
CTCCGATGGCCAGGATAATTTCACATTGGCGGGTGAGTCGAGTTGGAGTC
CTCCCTCCTCCGGGATGGGTGTGGAAAATGGGAAGGTTTCACCTCCCAAG
CCAAACTGCCTGGGAAACTTTATCTTACAGTTCTTGGTGATAAGATCTGCA
GTCGGCTTTGCCTGAAGAGGAAGAGGAGAGGAGGGGACACCAGCTAGGA
CAGAAGGGGCAGGGAGGAATAGAGATGGGGCAGAGGCACATTTAGAAAC
AACAAGGGTTGGTGACAAGACGTGAGGCAGGCTTGAGGGGAAAGCTTGC
TGATGAGTCCCAAATATGCTTTGCAGGGGGGGGGGGGGGGGAATCAAGG
CTGGAGAAGCAAGCAAGCAAGACAGCAAGACAGCGGGCGGGTAGTATGT
GGGAGCCAGCAGAAGCGCTTTGATTCACCGCTATCCTGGGCTCAATCCTC
TGGCCTCGCACTGGGGAAATGGGGTCTGAGTGGTCCTTGCTGTCTTCTGGC
AAAGGCTGCTGGGAGCAAAAGACTTCACAGGGCGTGAGAGGATTAACTTT
TCTGGTGAATTAAGCTTCTTGACATTTGCAGAACGTCAATGCCTTAAAATT
CTAGCTCTGAAGGAGAAGGGAATGAAGGGGAAAGAGGGAAGGTTGGTGT
GGAGAAATTCCCAAGCTTCTGGGGTGTAACACAGCTCCAGTCCCTACCCT

ATTGGGAAAGCCCAGACTCAGGAGACATGGTCCAAGGAAATCCCTGACA
GAAAACCGGGAGAGGGCAGGGCTGTGGAGCCTGAAACACACCCCACACC
CATGGTGACAGTCACTTCTCACATATGCCTAGGAACCTATCTGAAACCTTT
GGCCATCTCTCTCTGAAAAGATGAGGCTGCAAATACACACACACACACAC
ACACACACACACACACACACACACACACACACACACACACACACACAAA
TGTCCTTCAAGCCTTTTTGACAAGGTTTTCTGGTGGATCCCGGGGATATGA
AGTTGTTCTCAGCAGATATCTGGGAGTCTTGACTCCTGGCCCTCTGAGTAA
ATGGATGAAGCGAAGAAGAATGGGGTCCTCTGAGTAACAGGTGGATCTA
GAAAATCCTATAGGAGTCACCAGGGCACGGTGGAGGAGGGTAAGGTACA
GAACTAACAATAGCCCGAGAAGGGGAAACAGCAGGAGATGATTCCAGAG
ACGTAGTGACCCCAAGCTGCAAGGGAAAGCATGAGGGGCCAGCAGGAAG
GCCGACATGGCAGGTTGTCAGCTTCTAGATCGGAAGGCGGGTCACACTTG
CTCTTTCTATCCTCAGGGCCACCTCCTCGCACAAGGAGTGTCTCCTACATC
AACATCATCATGCCTTCAGTGTTTGGTACCATCTGTCTCCTGGGCATTGTG
GGAAACTCCACAGTCATTTTTGCCGTGGTGAAGAAATCCAAGCTGCACTG
GTGCAGCAACGTCCCTGACATCTTCATCATCAACCTCTCTGTGGTGGATCT
GCTTTTCCTGCTGGGCATGCCTTTCATGATCCACCAGCTCATGGGTAATGG
TGTCTGGCACTTTGGGGAAACCATGTGCACCCTCATCACAGCCATGGACG
CCAACAGTCAGTTCACCAGCACCTACATCCTGACTGCTATGGCCATTGACC
GCTACTTGGCCACCGTCCATCCCATCTCCTCCACCAAGTTCCGGAAGCCCT
CCATGGCCACCCTGGTGATCTGCCTCCTGTGGGCTCTCTCGTTCATTAGCA
TCACTCCTGTGTGGCTCTATGCCAGGCTTATCCCCTTCCCAGGGGGTGCTG
TGGGCTGTGGCATCCGCCTACCAAACCCAGATACTGATCTTTACTGGTTCA
CTCTGTATCAGTTTTTCCTGGCCTTCGCCCTTCCGTTTGTGGTCATCACTGC
TGCGTACGTGAAAATACTACAGCGCATGACGTCTTCGGTGGCCCCAGCCT
CTCAACGCAGCATCCGGCTTCGGACAAAGAGGGTGACCCGCACAGCCATT
GCCATCTGTCTGGTCTTCTTTGTGTGCTGGGCGCCCTACTACGTGCTGCAG
CTGACCCAGTTGTCCATCAGCCGCCCGACCCTCACATTCGTCTACCTGTAC
AATGCGGCCATCAGCTTGGGCTATGCCAACAGCTGCCTCAATCCCTTTGTG
TACATAGTACTCTGTGAGACCTTTCGAAAACGCTTGGTGCTGTCGGTGAA
GCCCGCGGCCCAGGGGCAGCTTCGCACGGTCAGCAATGCTCAGACAGCTG
ACGAGGAGAGGACAGAAAGCAAAGGCACCATGGTGAGCAAGGGCGAGG
AGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTA
AACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCT
ACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTG

CCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAG
CCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGC
CCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAAC
TACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACC
GCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGG
GCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCG
ACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACAT
CGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCC
ATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCA
GTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGC
TGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTAC
AAGTAA

SEQ. ID. NO. 28: Human short form/mouse species chimeric MCH1R-linker-EGFP

Nucleic acid sequence start codon and start and stop codons for mouse MCH1R and EGFP, respectively, are highlighted and a 12 amino acid linker sequence is denoted in lower case:

ATGGACCTGGAAGCCTCGCTGCTGCCCACTGGTCCCAATGCCAGCAACAC
CTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCTCCTCGCACGG
GGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTCGGCACCATCT
GCCTCCTGGGCATCATCGGGAACTCCACGGTCATCTTCGCGGTCGTGAAG
AAGTCCAAGCTGCACTGGTGCAACAACGTCCCCGACATCTTCATCATCAA
CCTCTCGGTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTTCATGATCCA
CCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCATGTGCACCC
TCATCACGGCCATGGATGCCAATAGTCAGTTCACCAGCACCTACATCCTG
ACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCCATCTCTTCC
ACGAAGTTCCGGAAGCCCTCCATGGCCACCCTGGTGATCTGCCTCCTGTG
GGCTCTCTCGTTCATTAGCATCACTCCTGTGTGGCTCTATGCCAGGCTTAT
CCCCTTCCCAGGGGGTGCTGTGGGCTGTGGCATCCGCCTACCAAACCCAG
ATACTGATCTTTACTGGTTCACTCTGTATCAGTTTTTCCTGGCCTTCGCCCT
TCCGTTTGTGGTCATCACTGCTGCGTACGTGAAAATACTACAGCGCATGAC
GTCTTCGGTGGCCCCAGCCTCTCAACGCAGCATCCGGCTTCGGACAAAGA
GGGTGACCCGCACAGCCATTGCCATCTGTCTGGTCTTCTTTGTGTGCTGGG
CGCCCTACTACGTGCTGCAGCTGACCCAGTTGTCCATCAGCCGCCCGACC

CTCACATTCGTCTACCTGTACAATGCGGCCATCAGCTTGGGCTATGCCAAC
AGCTGCCTCAATCCCTTTGTGTACATAGTACTCTGTGAGACCTTTCGAAAA
CGCTTGGTGCTGTCGGTGAAGCCCGCGGCCCAGGGGCAGCTTCGCACGGT
CAGCAATGCTCAGACAGCTGACGAGGAGAGGACAGAAAGCAAAGGCACC
gtcgacggtaccgcgggcccgggatccatcgccacc**ATG**GTGAGCAAGGGCGAGGAGCTGTT
CACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCC
ACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAA
GCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGC
CCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTAC
CCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGG
CTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGA
CCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAG
CTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGC
TGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAG
AAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACG
GCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGAC
GGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT
GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCG
TGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAG**TAA**

SEQ. ID. NO. 29: Human long form/mouse species chimeric MCH1R-linker-EGFP

Nucleic acid sequence start codon and start and stop codons for mouse MCH1R and EGFP, respectively, are highlighted and a 12 amino acid linker sequence is denoted in lower case:

ATGTCAGTGGGAGCCATGAAGAAGGGAGTGGGGAGGGCAGTTGGGCTTG
GAGGCGGCAGCGGCTGCCAGGCTACGGAGGAAGACCCCCTTCCCAACTGC
GGGGCTTGCGCTCCGGGACAAGGTGGCAGGCGCTGGAGGCTGCCGCAGC
CTGCGTGGGTGGAGGGGAGCTCAGCTCGGTTGTGGGAGCAGGCGACCGG
CACTGGCTGGATGGACCTGGAAGCCTCGCTGCTGCCCACTGGTCCCAACG
CCAGCAACACCTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCT
CCTCGCACGGGGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTC
GGCACCATCTGCCTCCTGGGCATCATCGGGAACTCCACGGTCATCTTCGCG
GTCGTGAAGAAGTCCAAGCTGCACTGGTGCAACAACGTCCCCGACATCTT
CATCATCAACCTCTCGGTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTT

CATGATCCACCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCA
TGTGCACCCTCATCACGGCCATGGATGCCAATAGTCAGTTCACCAGCACC
TACATCCTGACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCC
ATCTCTTCCACGAAGTTCCGGAAGCCCTCCATGGCCACCCTGGTGATCTGC
CTCCTGTGGGCTCTCTCGTTCATTAGCATCACTCCTGTGTGGCTCTATGCC
AGGCTTATCCCCTTCCCAGGGGGTGCTGTGGGCTGTGGCATCCGCCTACCA
AACCCAGATACTGATCTTTACTGGTTCACTCTGTATCAGTTTTTCCTGGCCT
TCGCCCTTCCGTTTGTGGTCATCACTGCTGCGTACGTGAAAATACTACAGC
GCATGACGTCTTCGGTGGCCCCAGCCTCTCAACGCAGCATCCGGCTTCGG
ACAAAGAGGGTGACCCGCACAGCCATTGCCATCTGTCTGGTCTTCTTTGTG
TGCTGGGCGCCCTACTACGTGCTGCAGCTGACCCAGTTGTCCATCAGCCGC
CCGACCCTCACATTCGTCTACCTGTACAATGCGGCCATCAGCTTGGGCTAT
GCCAACAGCTGCCTCAATCCCTTTGTGTACATAGTACTCTGTGAGACCTTT
CGAAAACGCTTGGTGCTGTCGGTGAAGCCCGCGGCCCAGGGGCAGCTTCG
CACGGTCAGCAATGCTCAGACAGCTGACGAGGAGAGGACAGAAAGCAAA
GGCACCgtcgacggtaccgcgggcccgggatccatcgccaccATGGTGAGCAAGGGCGAGGA
GCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAA
ACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTAC
GGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCC
CTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCC
GCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCC
GAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTA
CAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGC
ATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGC
ACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGAC
AAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCG
AGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATC
GGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTC
CGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGG
AGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAG
TAA

Example 2: Generation of Chimeric and Fusion Proteins

DNA vectors encoding fusion proteins between a MCH-R receptor (MCH1R) and several different superbright variants of Green Fluorescent Protein

(GFP) were generated. GFP variants were fused either via a 12 amino acid linker: TCGACGGTACCGCGGGCCCGGGATCCATCGCCACC (SEQ. ID. NO. 30), amino acid sequence: VDGTAGPGSIAT (SEQ. ID. NO. 31) (linker fusions) or directly to the C-terminus of MCH1R (direct fusions).

Mouse MCH1R-linker-GFP Variant Fusion Constructs

Initially, mouse MCH1R was fused in frame via the linker to Enhanced Green Fluorescent Protein (EGFP). MCH1R was PCR-amplified (95°C for 5 minutes; 95°C for 30 seconds, 60°C for 45 seconds, 68°C for 3.5 minutes, for 15 cycles; 68°C for 7 minutes) from a full-length mouse MCH1R genomic DNA lambda clone utilizing a high fidelity polymerase mix (Expand High Fidelity PCR System from Boehringer Mannheim) and PCR primers [MCH1R (Eco RI) 5': GCGAATTCACCATGGATCTGCAAGCCTCG (SEQ. ID. NO. 32), MCH1R (Sal I) 3': GCGTCGACGGTGCCTTTGCTTTCTGTCC (SEQ. ID. NO. 33)] that generated Eco RI and Sal I enzymatic restriction sites at the N- and C-terminus, respectively. The MCH1R N-terminal PCR primer was also designed to introduce a Kozak consensus sequence for translation which contained an Nco I site (5'-ACCATGG-3'), and the MCH1R C-terminal PCR primer was also designed to eliminate the endogenous stop codon present in the mouse MCH1R gene. The resulting PCR product was phenol/chloroform extracted, restriction digested with Eco RI and Sal I, gel purified, and subcloned in frame into the multicloning site of Clontech's pEGFP-N3 vector between Eco RI and Sal I sites. Several resulting clones for this construct were sequenced to identify a clone with an entirely correct nucleotide sequence. This clone was named mMCH1R-l-EGFP for mouse MCH1R-linker-EGFP.

An approximately 760 bp Sal I to Not I fragment of mMCH1R-l-EGFP was excised, gel purified, and subcloned into the multicloning site of pBluescript (SK+) (Stratagene) between Sall and Not I sites. An approximately 710 bp Nco I to Bsr G1 fragment of EGFP was excised from the resulting pBluescript-EGFP vector and replaced with the corresponding Nco I to Bsr G1 fragment of either Emerald, Topaz, or W1B (other superbright GFP variants), which were excised from vectors pRSET-Emerald, pRSET-Topaz, and pRSET-W1B, respectively. pRSET-Emerald, pRSET-Topaz, and pRSET-W1B were obtained from Aurora Biosciences Co. Sal I to Not I fragments containing either Emerald, Topaz, or W1B were excised from the resulting pBluescript-Emerald, pBluescript-Topaz, and pBluescript-W1B vectors, respectively. Appropriate fragments were gel purified and subcloned into mMCH1R-

l-EGFP digested with Sal I and Not I, replacing the Sal I to Not I EGFP fragment with the corresponding Sal I to Not I fragment from either Emerald, Topaz, or W1B. Several clones for each construct were sequenced to confirm the presence of the appropriate GFP variant. The resulting vectors were named mMCH1R-l-Emerald, mMCH1R-l-Topaz, and mMCH1R-l-W1B for mouse MCH1R-linker-Emerald, mouse MCH1R-linker-Topaz, and mouse MCH1R-linker-W1B, respectively.

Mouse MCH1R/GFP Variant Direct Fusion Constructs

A two step PCR strategy was employed to generate the direct fusion constructs. First, mouse MCH1R, EGFP, and Emerald were PCR-amplified from a full-length mouse MCH1R genomic DNA lambda clone, Clontech's pEGFP-N3 vector, and Aurora's pRSET-Emerald vector, respectively. Mouse MCH1R was PCR-amplified according to the previously mentioned conditions utilizing the same N-terminal PCR primer [MCH1R (Eco RI) 5': GCGAATTCACCATGGATCTGCA AGCCTCG (SEQ. ID. NO. 32)], but in this case a different C-terminal PCR primer was employed. The C-terminal PCR primer [MCH1R (EGFP/Emerald) 3': CCTTGCTCACCATGGTGCCTTTGCTTTCTGTCC (SEQ. ID. NO. 34)] eliminated the endogenous stop codon of mouse MCH1R as before and introduced a region of nucleotide sequence complementary to the nucleotide sequence of the N-terminus of EGFP.

EGFP and Emerald were PCR-amplified (95°C for 5 minutes; 95°C for 30 seconds, 60°C for 45 seconds, 68°C for 1.5 minutes, for 15 cycles; 68°C for 7 minutes) separately with a high fidelity polymerase mix (Advantage HF-2 from Clontech) from their respective templates utilizing a common N-terminal PCR primer [EGFP/Emerald (MCH1R) 5': CAGAAAGCAAAGGCACCATGGTGAGCAA GGGCGAGGAGC (SEQ. ID. NO. 35)] that generated a region of nucleotide sequence complementary to the C-terminus of mouse MCH1R and C-terminal PCR primers [EGFP 3': GGCGGATCCTCTAGAGTCGCGGCC (SEQ. ID. NO. 36), or Emerald (EGFP) 3': GCTCTAGAGTCGCGGCCGCTTACTTGTACAGCTCGTCC (SEQ. ID. NO. 37)] that generated a Not I site at the C-terminus. The resulting PCR products were electrophoresed on an agarose gel and the appropriate fragments were gel purified.

In a second PCR step, PCR reactions were set up between the previously generated mouse MCH1R and EGFP, or mouse MCH1R and Emerald PCR products. Following an initial 5 minute denaturation step at 95°C, two rounds of

thermocycling (95°C for 30 seconds, 60°C for 45 seconds, 68°C for 4 minutes) were performed in the absence of PCR primers. This allowed the mouse MCH1R and GFP variants to anneal at their complementary regions and to be filled in by the high fidelity polymerase mix (Expand High Fidelity PCR System from Boehringer Mannheim), yielding double stranded template DNA.

Subsequently, the common N-terminal mouse MCH1R [MCH1R (Eco RI) 5': GCGAATTCACCATGGATCTGCAAGCCTCG (SEQ. ID. NO. 32)] and appropriate C-terminal PCR primers [EGFP 3': GGCGGATCCTCTAGAGTC GCGGCC (SEQ. ID. NO. 36) or Emerald (EGFP) 3': GCTCTAGAGTCGCGG CCGCTTACTTGTACAGCTCGTCC (SEQ. ID. NO. 37)] were added to the reactions and thermocycling was continued for an additional fifteen cycles followed by a final extension at 68°C for 7 minutes. The resulting PCR products were phenol/chloroform extracted, restriction digested with Eco RI and Not I, electrophoresed on an agarose gel, and appropriate fragments were gel purified.

These Eco RI to Not I fragments represent direct fusions between either mouse MCH1R and EGFP, or mouse MCH1R and Emerald. Clontech's pEGFP-N3 vector was restriction digested with Eco RI and Not I liberating an approximately 780 bp Eco RI to Not I EGFP fragment. This restriction digest was electrophoresed on an agarose gel and the approximately 3.9 Kb pEGFP-N3 vector backbone was gel purified. Eco RI to Not I mouse MCH1R/EGFP or mouse MCH1R/Emerald direct fusion fragments were subcloned into the pEGFP-N3 vector backbone between Eco RI and Not I sites. Several resulting clones for each of these two constructs were sequenced to identify clones with correct nucleotide sequence; however, no clones with entirely correct nucleotide sequences were identified. Fortunately, several clones for each of the two constructs only had nucleotide mismatches in the intron region of mouse MCH1R, and therefore, were not expected to effect the functionality of the resulting fusion proteins. These clones were named mMCH1R/EGFP and mMCH1R/Emerald for mouse MCH1R/EGFP direct fusion and mouse MCH1R/Emerald direct fusion, respectively.

Human Short and Long Form/Mouse Species Chimeric MCH1R-linker-GFP Variant Fusion Constructs

The initial mouse MCH1R-linker-GFP variant fusion constructs were modified to generate both human short form and human long form/mouse species

chimeric MCH1R-linker-GFP variant fusion constructs. An approximately 1.7 kb Hind III to Bsp EI fragment of the mouse MCH1R gene containing exon 1, the intron, and 127 amino acids of exon 2 was excised from the various mouse MCH1R-linker-GFP variant fusion constructs and replaced by either an approximately 470 bp Hind III to Bsp EI fragment from the wild-type human MCH1R short form or an approximately 670 bp Hind III to Bsp EI fragment from the wild-type human MCH1R long form.

Several clones for each construct were sequenced to confirm the presence of the N-terminal region of either the human MCH1R short or long forms. These clones were named hshort/mMCH1R-l-GFP variant or hlong/mMCH1R-l-GFP variant for human short form/mouse species chimeric MCH1R-linker-GFP variant and human long form/mouse species chimeric MCH1R-linker-GFP variant, respectively.

Example 3: Functional Evaluation of MCH1R/GFP Variant Fusion Proteins

Both HEK293 Aequorin (National Institutes of Health) and CHO mammalian cell lines were transiently transfected with the various MCH1R/GFP variant fusion constructs, as well as the appropriate control constructs. Transfection was performed using Lipofectamine 2000 (Gibco BRL) per the manufacturer recommended protocol. Approximately 48 hours after transfection cells were harvested, stimulated with various concentrations of human MCH, and assayed for either aequorin bioluminescence (HEK293 Aequorin cells) or cAMP production (CHO cells). Aequorin bioluminescence is a representative measure of intracellular Ca^{2+} mobilization. cAMP production was measured with the Adenylyl Cyclase Activation FlashPlate Assay (NEN Life Science Products, Inc.).

Following transient transfection of the mMCH1R-linker-EGFP construct (MCH-R-l-EGFP) into HEK293 Aequorin cells, the resulting fusion protein exhibited functional activity comparable to that of the wild-type human MCH1R short form (MCH-R wt). By this functional assay, the EC_{50} value for mMCH1R-l-EGFP was nearly identical to that of the wild-type human short form receptor (Figure 1).

Following transient transfections of the mMCH1R-l-EGFP and mMCH1R/EGFP fusion constructs into CHO cells, the resulting fusion proteins exhibited functional activity comparable to that of the wild-type human MCH1R short form. By this functional assay, the EC_{50} values for mMCH1R-l-EGFP and mMCH1R/EGFP were comparable to that of the wild-type human receptor (Table 1).

Transient transfections with the corresponding Emerald constructs yielded similar results (data not shown).

Table 1

Receptor	EC_{50} (nM)
Wild-type Human MCH1R Short Form	2.166
Mouse MCH1R/EGFP	0.819
Mouse MCH1R-l-EGFP	3.199

Following transient transfections of the human short form/mouse species chimeric MCH1R-l-EGFP (HuShort/mMCH1R-l-EGFP) and human long form/mouse species chimeric MCH1R-l-EGFP (HuLong/mMCH1R-l-EGFP) constructs into HEK293 cells, the resulting fusion proteins exhibited functional activity comparable to that of the wild-type human MHC1R short and long forms, respectively. By this functional assay, the EC_{50} value for each fusion proteins was nearly identical to that of the corresponding wild-type human receptor (Table 2).

Table 2

Receptor	EC_{50} (nM)
Wild-type Human MCH1R Short Form	22.27
HuShort/mMCH1R-l-EGFP	19.54
Wild-type Human MCH1R Long Form	196.7
HuLong/mMCH1R-l-EGFP Form	217.5

Following transient transfections of the human short form/mouse species chimeric MCH1R-l-EGFP (HuShort/mMCH1R-l-EGFP) and human long form/mouse species chimeric MCH1R-l-EGFP (HuLong/mMCH1R-l-EGFP) constructs into CHO cells, the resulting fusion proteins exhibited functional activity comparable to or less than that of the wild-type human MHC1R short and long forms, respectively (Table 3). By this functional assay, the EC_{50} value for the human short form/mouse species chimeric MCH1R-l-EGFP fusion protein was comparable to that of the corresponding wild-type human receptor, whereas, the human long form/mouse

species chimeric MCH1R-l-EGFP fusion protein had an EC_{50} value approximately 7.5-fold higher than that of its corresponding wild-type control.

Table 3

Receptor	EC_{50} (nM)
Wild-type Human MCH1R Short Form	1.029
Wild-type Human MCH1R Long Form	1.515
HuShort/mMCH1R-l-EFGP	1.565
HuLong/mMCH1R-l-EGFP	11.580

Transient expression of all the MCH1R/GFP variant fusion proteins that underwent functional evaluation resulted in fluorescence primarily associated with the plasma membrane in both HEK293 and CHO cells (data not shown). This pattern of fluorescence is consistent with a predominant membrane associated localization.

Example 4: Generation of Stable Cell Lines

Wild-type CHO cells were transfected using SuperFect (Qiagen) and either mouse MCH-1R-EGFP or human short/mouse species chimeric MCH-1R-EGFP. Forty-eight hours after transfection, transfected cells were subjected to positive selection for approximately ten days in media containing G418. Following selection, MCH-1R-EGFP expressing CHO cells were bulk sorted by Fluorescence Assisted Cell Sorting (FACS) for one or two rounds on the basis of fluorescence intensity to increase the population of cells expressing EGFP. Following bulk sorts, individual clones of varying fluorescence intensities were isolated by FACS and expanded.

Fluorometric Microvolume Assay Technology (FMAT) was initially employed to screen a large number of stable clones by whole cell binding with a fluorescently labeled MCH derivative (SymJz-MCH, PE Biosystems) to identify those clones with good specific binding windows. Several clones exhibiting specific binding windows greater than 3-fold were further evaluated for MCH binding with the SPA-based Binding Assay. Cells from individual clones were dissociated in enzyme free dissociation media and cell membranes were prepared and subsequently tested for

their ability to bind [^{125}I]Phe^{13}Tyr^{19}-MCH in the presence of human MCH. CHO cell lines expressing either mouse MCH-1R-EGFP or human short/mouse species chimeric MCH-1R-EGFP (Figure 4) displayed IC50 values with MCH that were indistinguishable from the corresponding IC50 values obtained with a CHO cell line expressing the wild-type human short isoform of MCH-1R.

The functional activity of these clones was evaluated with the cAMP Flashplate Assay (Figures 2 and 3). CHO cell lines expressing either mouse MCH-1R-EGFP (Figure 2) or human short/mouse species chimeric MCH-1R-EGFP (Figure 3) displayed EC50 values with human MCH that were indistinguishable from the EC50 value obtained with a CHO cell line expressing the wild-type human short isoform of MCH-1R.

The subcellular localization of the MCH-1R-EGFP fusion proteins were determined by confocal microscopy utilizing EGFP fluorescence as a marker for MCH-1R expression. CHO cell lines stably expressing either mouse MCH-1R-EGFP or human short/mouse species chimeric MCH-1R-EGFP displayed EGFP fluorescence primarily associated with the plasma membrane, demonstrating that these MCH-1R-EGFP fusion proteins are primarily associated with the plasma membrane.

Other embodiments are within the following claims. While several embodiments have been shown and described, various modifications may be made without departing from the spirit and scope of the present invention.

WHAT IS CLAIMED IS:

1. A fusion protein comprising:

a) a melanin concentrating hormone receptor polypeptide region comprising a sequence selected from the group consisting of: SEQ. ID. NO. 1, SEQ. ID. NO. 2, SEQ. SEQ. ID. NO. 3, SEQ. ID. NO. 4, and SEQ. ID. NO. 5; and

b) a fluorescent polypeptide region joined directly, or though a linker, to the carboxy side of said melanin concentrating hormone receptor polypeptide region.

2. The protein of claim 1, wherein said fluorescent polypeptide region consists of an amino acid sequence selected from the group consisting of SEQ. ID. NO. 6, SEQ. ID. NO. 7, SEQ. ID. NO. 8, SEQ. ID. NO. 9, and SEQ. ID. NO. 10.

3. The protein of claim 2, wherein said melanin concentrating hormone polypeptide region consists of a sequence selected from the group consisting of: SEQ. ID. NO. 1, SEQ. ID. NO. 2, SEQ. ID. NO. 3, SEQ. ID. NO. 4, and SEQ. ID. NO. 5.

4. The protein of claim 3, wherein said protein consists essentially of said melanin concentrating hormone receptor polypeptide region and said fluorescent polypeptide region.

5. The protein of claim 4, wherein said protein consists of the amino acid sequence of SEQ. ID. NO. 11 or SEQ. ID. NO. 12.

6. The protein of claim 1, wherein said melanin concentrating hormone polypeptide region is a chimeric polypeptide comprising (a) an MCH binding region from a first species and (b) a transmembrane and intracellular domain region from a second species joined directly, or though a linker, to the carboxy side of said MCH binding region.

7. The protein of claim 6, wherein said fluorescent polypeptide region consists of an amino acid sequence selected from the group consisting of: SEQ. ID. NO. 6, SEQ. ID. NO. 7, SEQ. ID. NO. 8, SEQ. ID. NO. 9, and SEQ. ID. NO. 10.

8. The protein of claim 7, wherein said protein consists of the amino acid sequence of SEQ. ID. NO. 13 or SEQ. ID. NO. 14.

9. A chimeric melanin concentrating hormone protein comprising:

a) a melanin concentrating hormone binding region characteristic of a human melanin concentrating hormone receptor;

b) a transmembrane domain characteristic of a non-human melanin concentrating hormone receptor; and

c) an intracellular domain characteristic of a non-human melanin concentrating hormone receptor.

10. The protein of claim 9, wherein said protein comprises a melanin concentrating hormone receptor polypeptide having a sequence similarity of at least 75% with either SEQ. ID. NO. 4 or SEQ. ID. NO. 5.

11. The protein of claim 10, wherein said protein comprises the sequence of SEQ. ID. NO. 4 or SEQ. ID. NO. 5.

12. The protein of claim 11, wherein said protein consists of the sequence of SEQ. ID. NO. 4 or SEQ. ID. NO. 5.

13. A nucleic acid comprising a nucleotide sequence encoding for the protein of claim 1.

14. The nucleic acid of claim 13, wherein said nucleotide sequence is a contiguous sequence.

15. The nucleic acid of claim 13, wherein said nucleotide sequence is selected from the group consisting of SEQ. ID. NO. 26, SEQ. ID. NO. 27, SEQ. ID. NO. 28 and SEQ. ID. NO. 29.

16. A nucleic acid comprising a nucleotide sequence encoding for the protein of claim 9.

17. The nucleic acid of claim 16, wherein said nucleotide sequence is a contiguous sequence.

18. The nucleic acid of claim 16, wherein said nucleotide sequence is selected from the group consisting of SEQ. ID. NO. 19 and SEQ. ID. NO. 20.

19. An expression vector comprising the nucleic acid of claim 13.

20. An expression vector comprising the nucleic acid of claim 16.

21. A recombinant cell comprising the nucleic acid of claim 13.

22. The recombinant cell of claim 21, wherein said nucleic acid is present in an expression vector.

23. The recombinant cell of claim 21, wherein said nucleic acid is present in the genome of said cell.

24. A recombinant cell comprising the nucleic acid of claim 16.

25. The recombinant cell of claim 24, wherein said nucleic acid is present in an expression vector.

26. The recombinant cell of claim 24, wherein said nucleic acid is present in the genome of said cell.

27. A non-human transgenic animal comprising the nucleic acid of claim 13.

28. A non-human transgenic animal comprising the nucleic acid of claim 16.

29. A method for assaying for melanin concentrating hormone receptor active compounds comprising the steps of:

a) contacting the cell of claim 21 with a test preparation comprising one or more test compounds; and

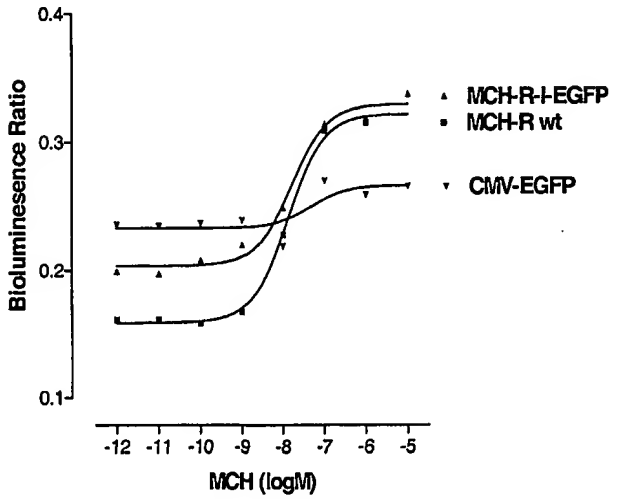
b) measuring the effect of said test preparation on one or more melanin concentrating hormone receptor activities.

30. A method for assaying for melanin concentrating hormone receptor active compounds comprising the steps of:

a) contacting the cell of claim 24 with a test preparation comprising one or more test compounds; and

b) measuring the effect of said test preparation on one or more melanin concentrating hormone receptor activities.

mMCH1R-l-EGFP Aequorin Assay



MCH-R wt	MCH-R-l-EGFP
1.3020e-008	1.6060e-008

Fig. 1

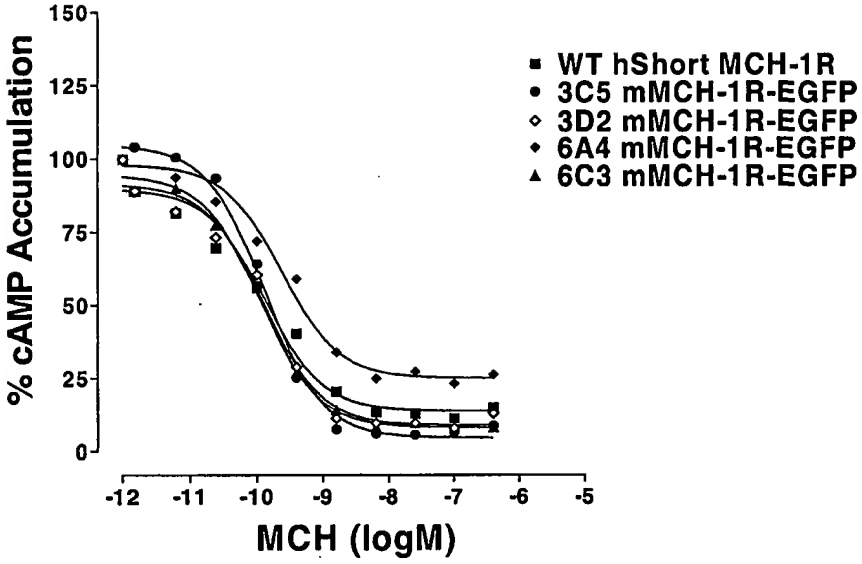

% cAMP Accumulation
150
125
100
75
50
25
0
-12
-11
-10
-9
-8
-7
-6
-5
MCH (logM)
WT hShort MCH-1R
3C5 mMCH-1R-EGFP
3D2 mMCH-1R-EGFP
6A4 mMCH-1R-EGFP
6C3 mMCH-1R-EGFP

Fig. 2

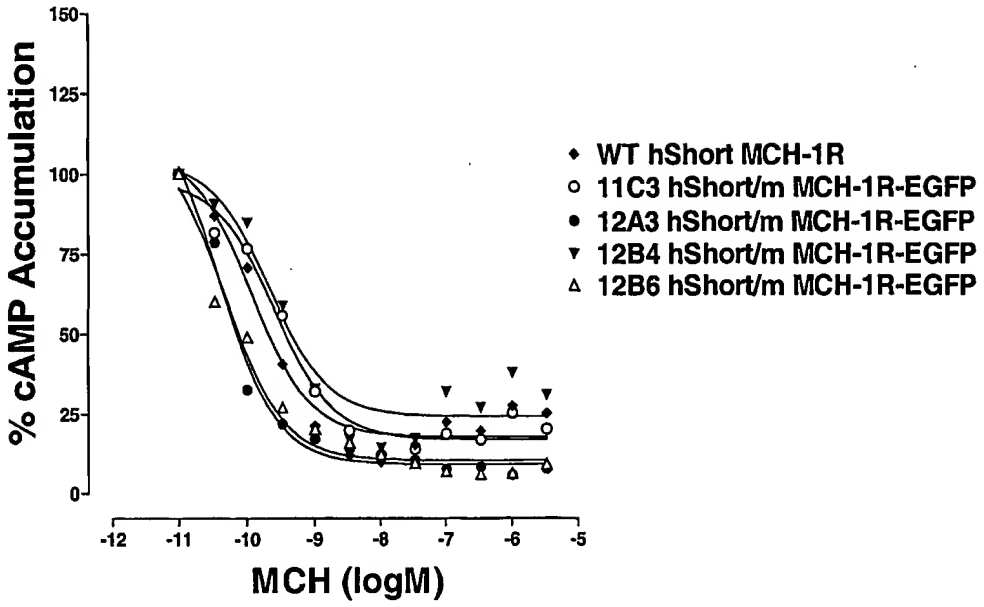

% cAMP Accumulation
150
125
100
75
50
25
0
-12
-11
-10
-9
-8
-7
-6
-5
MCH (logM)
WT hShort MCH-1R
11C3 hShort/m MCH-1R-EGFP
12A3 hShort/m MCH-1R-EGFP
12B4 hShort/m MCH-1R-EGFP
12B6 hShort/m MCH-1R-EGFP

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/08071

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :C07K 14/72 , 19/00; C12N 15/62
US CL : 435/69.7, 252.3, 320.1; 530/350; 536/23.4
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.7, 252.3, 320.1; 530/350; 536/23.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, MEDLINE
search terms: fluores?, receptor#, green, g protein, melanin concentrating hormone receptor#

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NELSON et al. Characterization of an Intrinsically Fluorescent Gonadotropin-Releasing Hormone Receptor and Effects of Ligand Binding on Receptor Lateral Diffusion. Endocrinology. February 1999. Vol. 140. No. 2. pages 950-957, see entire document.	1-30
Y	AWAJI et al. Real-Time Optical Monitoring of Ligand-Mediated Internalization of alpha1b-Adrenoreceptor with Green Fluorescent Protein. Molecular Endocrinology. August 1998. Vol. 12. No. 8. pages 1099-1111, see entire document.	1-30

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
21 JUNE 2001	03 JUL 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JOHN D. ULM Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/08071

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BACHNER et al. Identification of Melanin Concentrating Hormone (MCH) as the Natural Ligand for the Orphan Somatostatin-Like Receptor 1 (SLC-1). FEBS Letters. 03 September 1999. Vol. 457. No.3. pages 522-524, see entire document.	1-30
Y	SALRO et al. Molecular Characterization of the Melanin-Concentrating-Hormone Receptor. Nature. 15 July 1999. Vol. 400. pages 265-269, see entire document.	1-30